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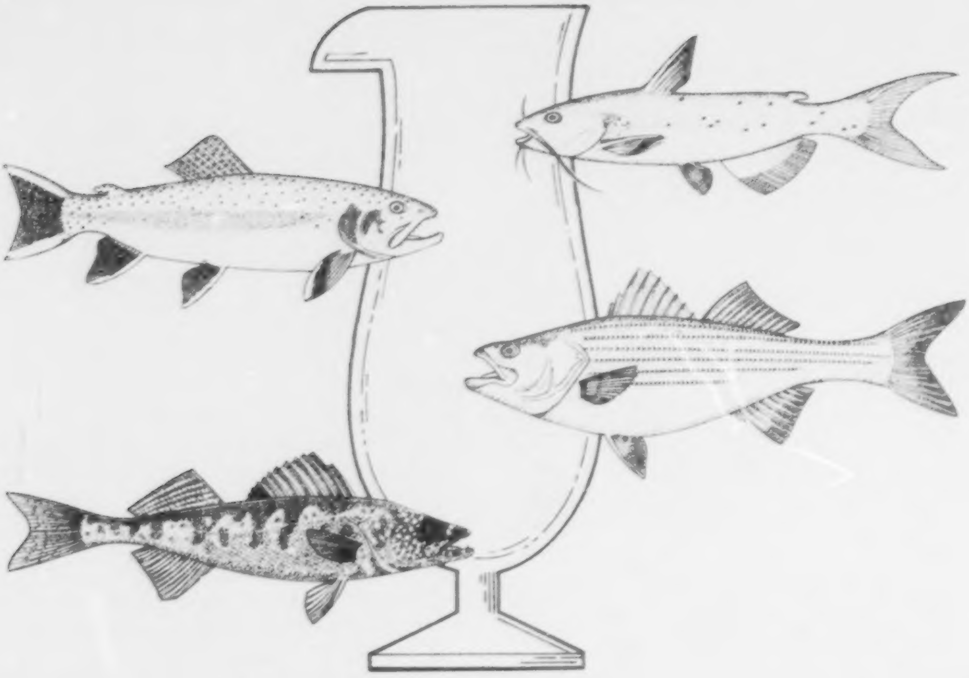

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Status of Fishery Chemicals in 1985

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Abstract—A summary list of registered or approved products is provided for fishery uses, effective June 1985. The current status of efforts to register others and a discussion of the need for new products is presented.

During the 6 years since the 1979 report on the registration status of fishery chemicals, priorities and needs for fishery compounds have changed significantly (Anonymous 1979). Some new products have been registered or approved, and a number of registered compounds have been cancelled or dropped and are no longer available. A *registered* compound is an available commercial product bearing an Environmental Protection Agency (EPA) or Food and Drug Administration (FDA) label specifying its allowed uses. An *approved* product does not necessarily have an EPA or FDA label, because some other classification or designation may allow its use in aquatic situations. Examples are salt, acetic acid, and carbon dioxide. This report provides a summary list of the products that were registered or approved by EPA or FDA for fishery uses effective June 1985, describes the current status of registration efforts, and discusses the need for new products in fisheries.

Current Registration Status

Confusion exists as to which chemicals have been approved or registered for aquatic use, which commercial products are approved for aquatic applications, and what use patterns are permitted. The U.S. Congress charged EPA with control of the use of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act and its amendments (Office of the Federal Register 1984b). The FDA was assigned the responsibility for regulating the use of drugs under the Federal Food, Drug, and Cosmetic Act and its amendments (Office of the Federal Register 1984a). The promulgation of policy, rules, and guidelines to implement these laws was also given to these respective agencies. Regulatory control covers testing, development, distribution, and use, as well as the manufacturing process, bulk shipment, formulation, retail packaging, labeling, interstate shipment, applicators, application rates, use patterns, and the species of fish on which the compound can be used.

Recently, both EPA and FDA have increased their efforts to enforce regulations that govern the use of drugs and chemicals in fish culture. Inspectors have visited hatcheries and fish farms to question the use of particular compounds. If the use is not consistent with an approved label or is not covered by another form of approval, the user may be subject to disciplinary action. Unfortunately, fish culturists sometimes purchase generic products rather than registered compounds in an effort to save money—a practice that may prevent sponsors from recovering the costs of registering their product and could cause them to remove the product from the market. Permits (Forms FDA 1800 Medicated Feed Application and FDA 2656 Registration of Drug Establishment) are required by FDA if fish culturists produce their own medicated feeds; quality assurance data must be provided to prove that the product is consistent with permitted drug levels. The discharge of water from fish cultural facilities is controlled by EPA under the National Pollutant Discharge Elimination System. Discharge permits require that each facility provide information on the types and amounts of chemicals discharged, and operate in accordance with specified effluent limitations, monitoring requirements, and other conditions.

Some unregistered uses of chemicals may be allowed by EPA if certain requirements are met. EPA does not regulate compounds such as sodium cyanide and isobornyl thiocyanoacetate (Thanite) as pesticides if they are used by state and federal biologists to collect fish for experimental purposes. However, state, local, or federal toxic substance regulations may restrict their use. Sodium cyanide must be used with extreme caution—never around domestic water supplies, and always in well ventilated areas and under close supervision. Insecticides that are registered for mosquito control in aquatic environments may also be used to control other aquatic nuisance organisms if (1) the pesticide is applied at concentrations or frequencies that are less than those stated on the label, (2) use on the target pest is not expressly prohibited, (3) the application is compatible with, and nontoxic to, the crop, animal, or site specified on the label, and (4) the method of application is not prohibited. Pesticides that meet these requirements include BTI (*Bacillus thuringiensis* Berliner variety *israelensis*), methoprene (Altosid), mineral oils, naled (Dibrom), parathion, petroleum oils, and solvents. Other insecticides are also registered to control mosquitos in aquatic situations, but these may be toxic to fish. They include chlorpyrifos (Dursban), dichlorvos (DDVP), fenthion (Baytex), lindane (BHC), malathion (Carbophos), methoxychlor (Methoxcide), pyrethroids, and temephos (Abate). Users are cautioned to read the label carefully to observe all prohibited uses and to follow all directions concerning methods of application.

The FDA uses two categories, *food fish* and *nonfood fish*, in determining which fishery use patterns are to be permitted. Food fish are species that, at some stage in their life cycle, may be consumed by humans, and include such species as black bass (*Micropterus* spp.), bluegills (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), salmon (*Oncorhynchus* spp. and *Salmo salar*), and trout (*Salmo* spp. and *Salvelinus* spp.). The regulations for food fish apply to all life stages, including eggs, fry, fingerlings, subadults, and brood fish. Nonfood fish are bait fishes such as golden shiners (*Notemigonus crysoleucas*), goldfish (*Carassius auratus*), and fathead minnows (*Pimephales promelas*), and the many species of ornamental fishes reared by aquarists. Although extensive testing for efficacy, animal safety, and environmental safety is required for both food and nonfood fishes, additional data on metabolites, residues, and residue persistence must be submitted for the establishment of a minimum tolerance and withdrawal time for chemicals to be applied to food fishes.

Registration Needs

Over 30 drugs and chemicals are registered or approved for use in fish culture and fishery management. However, new products are still needed, including a fungicide to replace malachite green; a broad spectrum antibiotic; controls for bacterial gill disease, viruses, copepods, and external protozoans, especially *Ichthyophthirius*, known as Ich; anesthetics with zero withdrawal times or no residues; new fish control agents and known selective toxicants, such as squoxin; selective toxicants for the rusty crayfish and Asiatic clam; and more effective aquatic herbicides.

Several agencies and chemical companies are investigating new drugs or additional uses of drugs or chemicals for fishery or aquatic situations. Screening programs are being conducted by the U.S. Fish and Wildlife Service to find a replacement for malachite green because it has been shown to be a potential teratogen in laboratory animals. So far, 15 possible replacement chemicals have been identified that show some promise for controlling fungal infections and nine have shown potential for controlling protozoan infections such as Ich. Romet-30 was registered in November 1984 for the control of furunculosis in salmonids, but it is also known to be effective against other bacteria. Erythromycin, sulfamerazine, and sulfamethazine are being investigated for possible use in controlling vertical transmission (within the eggs of salmon) of bacterial kidney disease. Oxolinic acid is being tested for use in controlling furunculosis, enteric redmouth disease, and vibriosis of salmonids. Tiamulin is a potential candidate to control both gram-negative and gram-positive bacteria. Candidate compounds being considered for possible control of bacterial gill disease in salmonids include chloramine-T and the hyamines

3500 and 1622. A project was recently begun at the La Crosse National Fishery Research Laboratory to identify the need for better anesthetics, and to delineate the activity of known fish anesthetics. The laboratory will then determine which anesthetics have the best potential for registration and initiate an effort to register them. A private company is apparently close to obtaining a registration for fluridone (Sonar), a herbicide with aquatic uses.

Efforts to register certain chemicals have been terminated because of their potential toxicity to the environment, human safety problems, lack of sufficient efficacy, or low priority. Four of the compounds dropped from consideration are known or suspected carcinogens: furazolidone (NF-180), nitrofurazone (Furacin), carofur, and chloramphenicol (Chloromycetin). The registration of silvex was cancelled because it has been shown to be a carcinogen. Use of acrolein (Aqualin) was discontinued by its United States sponsor because it was considered too hazardous for use. Diuron, Thanite, GD-174, toxaphene, ammonia, acriflavin, and sodium cyanide have also been dropped from consideration for registration.

Registered or Approved Compounds

Information is presented in the Appendix on products that have been registered or approved for use in fish culture or for the management of fishery resources. The Appendix tables are organized according to the following categories: therapeutants (Table A-1), anesthetics (Table A-2), disinfecting agents (Table A-3), water treatment compounds (Table A-4), herbicides and algicides (Table A-5), and fish control agents (Table A-6). Each table provides information on the product, such as trade and common names, sponsor, fishery use patterns, rates of application, and status of tolerance establishment. Comments are also provided on food use classifications, withdrawal times, and special conditions for use. Tables A-7 and A-8 provide conversion units and abbreviations. Readers and users are advised to always consult labels on formulations for specific details.

Several terms or phrases used in the tables should be noted because they have very specific meanings as defined in laws or in implementing regulations. *Tolerance* refers to residue levels of a drug or chemical that are permitted by regulatory agencies in food eaten by humans. *Withdrawal time* is the period of time that must pass after the last treatment or exposure to a certain drug, chemical, or pesticide before an animal can be consumed. Substances *generally recognized as safe* (GRAS) are widely used in items that FDA, after evaluating the existing data, has found to be safe for use in human food. (Examples are spices, oils, synthetic flavorings, and preservatives.) A *new animal drug application* (NADA) is a petition from a sponsor to the FDA requesting approval of the use of a drug for a specified purpose. When FDA grants approval, a sponsor is said to hold an approved NADA on that particular drug.

The *data call-in* program requires registrants of pesticides to provide EPA with needed data that can be used to assess each pesticide during the periodic reregistration process or to resolve special safety concerns. The purpose of the program is to ensure that adequate data are available or are collected before a pesticide is assessed for reregistration or other purposes. The *registration standards* program is EPA's approach to the assessment and reregistration of pesticide products as mandated by the U.S. Congress. The assessment involves a thorough review of the scientific data base underlying pesticide registrations and an identification of essential but missing scientific studies. Each registration standard document explains EPA's regulatory position on the use of a pesticide, analyzes available data, describes the rationale for EPA's position, and states the conditions that must be met to obtain reregistration. The *special review* program, formerly "rebuttable presumption against registration" (RPAR), is the process whereby EPA evaluates the history of use of a pesticide against established risk criteria. If the use poses hazards that clearly exceed the risk criteria, the registration may be cancelled. When the potential hazard has not been well defined or substantiated, EPA asks the sponsor to submit data to refute or rebut the stated concerns. If the review shows that no real hazard exists, the use is returned to the normal registration process. As the result of a special review program by EPA, a pesticide can be returned to the registration process, cancelled, restricted in use, or continued in administrative hearings.

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Appendix

In the Appendix Tables A-1 to A-6, products are listed by trade names; common names are in parentheses. Tables A-7 and A-8 give conversion formulas and nonstandard abbreviations, respectively.

TABLE A-1.—Therapeutants registered or approved for aquatic or fishery uses.

Product	Sponsor	Fishery use	Tolerance	Comments
Acetic acid, commercial grade (Vinegar)		Parasiticide—1,000–2,000 ppm for 1–10 min	Exempted from tolerance	Food fish use; declared as GRAS by FDA as general purpose food additive
Formalin-F (Formalin)	Natchez Animal Supply Co., Natchez, Mississippi	Parasiticide for use on trout, salmon, catfish, largemouth bass, and bluegill—25 ppm in ponds; up to 250 ppm for 1 h in tanks and raceways Fungicide for use on eggs of trout, salmon, and esocids—1,000–2,000 ppm for 15 min in egg treatment tanks	Not yet determined	Food fish use
Furanace capsules (Nifurpyrinol, Furpyridinol; P-7138)	Amdal Co., Division of Abbott Laboratories, North Chicago, Illinois	Antibacterial drug against columnaris disease in aquarium fish—3.8 mg capsule to 10 gal of water for 1 h	None established	Nonfood fish use only; do not use in saltwater aquariums or while egg or live-bearing fish are reproducing
Masoten (Trichlorfon)	Mobay Chemical Corp., Kansas City, Missouri	Parasiticide against anchorworms, lice, and gill flukes on goldfish or bait fish—0.25 ppm active ingredient for indefinite period	None established	Nonfood fish use only; not for use in streams; do not apply to ponds used as a source of drinking water for humans or animals; removed from pre-RPAR review and returned to reregistration process
Romet-30 (Ro-5: sulfadimethoxine + ormetoprim)	Hoffmann-La Roche, Inc., Nutley, New Jersey	Antibacterial drug against furunculosis on salmonids—50 mg/kg of fish per day for 5 d	0.1 ppm in salmonids	Food fish use; do not treat within 6 weeks of marketing or release as stocked fish
Salt (Sodium chloride)		Osmoregulatory enhancer—0.5–1% for indefinite period; 3% for 10–30 min	Exempted from tolerance	Food fish use; declared as GRAS by FDA
Sulfamerazine in fish grade (Sulfamerazine)	American Cyanamid Co., Princeton, New Jersey	Antibacterial drug against furunculosis on salmonids—10 g/100 lb of fish per day for 14 d in feed; discontinue use after 14 d	Zero tolerance in uncooked edible tissues of trout	Food fish use; do not treat within 3 weeks of marketing or stocking in stream open to fishing
Terramycin for fish (Oxytetracycline)	Pfizer, Inc., New York, New York	Antibacterial drug against *Aeromonas, Hemophilus,* and *Pseudomonas*—2.5–3.75 g/100 lb of fish per day for 10 d in feed	0.1 ppm in salmonids and catfish	Food fish use; 21-d preslaughter withdrawal

TABLE A-2.—Anesthetics registered or approved for aquatic or fishery uses.

Product	Sponsor	Fishery use	Tolerance	Comments
Carbonic acid (Carbon dioxide)		Anesthetic—200–400 ppm for 4 min	Exempted from tolerance	Declared as GRAS by FDA as general purpose food additive
Finquel (MS-222; tricaine methanesulfonate)	Argent Chemical Laboratories, Redmond, Washington	Anesthetic—50–330 ppm for 1–40 min Sedation—15–66 ppm for 6–48 h	None established	Food fish use; 21-d withdrawal after use before harvesting fish for food
Sodium bicarbonate (Baking soda)		Anesthetic—142–642 ppm for 5 min	Exempted from tolerance	Declared as GRAS by FDA as general purpose food additive

TABLE A-3.—Disinfecting agents registered or approved for aquatic or fishery uses.

Product	Sponsor	Fishery use	Tolerance	Comments
Net-Dip (Didecyl dimethyl ammonium chloride) [Distributed as Sanaqua by Aquavet, Hayward, California]	Aquasciences Research Group, Inc., North Kansas City, Missouri	Disinfection of aquarium and fish-holding equipment—2 fl oz in 4 gal water for 10 min Disinfection in fish disease control institutions—3.5 fl oz in 4 gal for 10 min	None established	Nonfood fish use; do not use directly on fish or other cultured aquatic life
Olin HTH Dry Chlorinator Granular (Calcium hypochlorite)	Olin Corp., Stamford, Connecticut	Disinfectant and sanitizer— 200 ppm available chlorine for 1 h to sanitize fish tanks, raceways, and utensils 5–10 ppm residual chlorine for 12–24 h to control algae and bacteria in fish ponds	Exempted from tolerance	Food fish use; to control algae or kill bacteria in fish ponds, remove all fish from pond before treatment

TABLE A-4.—Water treatment compounds registered or approved for aquatic or fishery uses.

Product	Sponsor	Fishery use	Tolerance	Comments
Fluorescein sodium		Dye to check water flows or dilution—0.1 ppm	None established	Food fish use; exempted from registration by EPA
Lime (Calcium hydroxide; calcium oxide; calcium carbonate)		Pond sterilant—1,338 lb/acre of quick lime; 1,784 lb/acre of slaked lime	Exempted from tolerance	Food fish use; declared as GRAS by FDA as general purpose food additive; EPA issued a data call-in letter in March 1983 and it was referred to a registration standard
Potassium permanganate		Oxidizer and detoxifier—2 ppm	None established	Food fish use; exempted from registration by EPA
Rhodamine B and WT		Dye to check water flows or dilution rates—20 ppb	Exempted from tolerance	Food fish use; exempted from registration by EPA

TABLE A-5.—Herbicides and algicides registered or approved for aquatic or fishery uses.

Product	Sponsor	Fishery use	Tolerance	Comments
Acme Norosac G-10 (Dichlobenil)	PBI/Gordon Corp., Kansas City, Missouri	Herbicide—70–150 lb/acre	None established	Nonfood fish use only; do not apply to water used for irrigation, livestock, or human consumption; do not use fish from treated water for 90 d after application; do not use in commercial fish or shellfish water
Agway Copper Sulfate (Copper sulfate)	Agway, Inc., Syracuse, New York	Algicide—2.75–5.5 lb/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; do not use water for any purpose for 7 d after treatments; do not use in trout ponds; if algae are visible, treat no more than ⅓ to ½ of the total pond at one time and wait 10–14 d between treatments to protect fish; trout and other species of fish may be killed at application rates, but fish toxicity generally decreases when the hardness of the water increases
Algae Control (Copper sulfate)	Athea Laboratories, Inc., Milwaukee, Wisconsin	Herbicide and algicide—0.25–2 lb/h for each ft^3/s	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; trout and certain other species of fish may be killed at application rates recommended, especially in soft or acid waters; however, fish toxicity generally decreases when the hardness of the water increases; consult state fish and game agencies before application, especially in public waters
Algaetrol-76 (Copper, elemental)	Thompson-Hayward Chemical Co., Kansas City, Kansas	Algicide—0.75–1.5 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; treated areas may be used immediately after treatment for swimming or fishing; do not use in water containing trout if the carbonate hardness of water is less than 50 ppm; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait 10–14 d between treatments
Algimycin PLL (Copper, elemental)	Great Lakes Biochemical Co., Inc., Milwaukee, Wisconsin	Algicide—4 oz/1,000 gal	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; remove large masses of floating algae manually before treatment if fish are present in the pond because decaying algae may reduce the oxygen content to a dangerous level for fish
Algimycin PLL-C (Copper, elemental)	Great Lakes Biochemical Co., Inc., Milwaukee, Wisconsin	Algicide—1–3 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; remove large masses of floating algae manually before treatment if fish are present in the pond because decaying algae may reduce the oxygen content to a dangerous level for fish; to minimize hazard to fish, never treat more than ⅓ of the surface at one time and wait 72 h before applying to another section
Algimycin PLL-C Slow Release (Copper sulfate)	Great Lakes Biochemical Co., Inc., Milwaukee, Wisconsin	Algicide—10–20 lb/acre-ft 20–40 lb/acre	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; if algae are visible, treat no more than ⅓ to ½ of the total pond at one time and wait 10–14 d between treatments to protect fish
Algon Algaecide (Copper, elemental)	Sungro Chemicals, Inc., Los Angeles, California	Algicide—1.3–2.5 gal/15,000 ft^3	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; may be toxic to trout and other species of fish; do not use in water containing trout if the carbonate hardness of water does not exceed 50 ppm; to minimize hazard to fish, treat ⅓ to ½ water area in a single operation and wait at least 10–14 d between treatments

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
Amitrol-T Liquid Herbicide (Amitrole)	Union Carbide Agricultural Products Co., Inc., Research Triangle Park, North Carolina	Herbicide— 0.5–10 gal/5–400 gal water	None established	Nonfood fish use only; for use in marshes and drainage ditches; do not apply where water will be used for irrigating, drinking, fishing, or other domestic purposes; a registration standard was issued on 30 March 1984 on its use as a herbicide; EPA is initiating a special review of amitrole because it causes cancer in laboratory animals
Aquacide (2,4-D)	Aquacide Co., St. Paul, Minnesota	Herbicide— 2.5–4 lb/1,000 ft^2	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; to avoid fish suffocation when treating an entire body of water, treat ⅓ to ½ of the area at a time and allow 3–5 weeks before continuing to untreated areas; water drawn directly from treated areas may damage desirable plants; consult state fish and game agencies before applying to public waters
Aqua-Clear (Diquat dibromide)	I. Schneid, Inc., Atlanta, Georgia	Herbicide and algicide— 0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; if dense weed areas are present, treat ⅓ to ½ of the water area in a single operation and wait at least 10–14 d between treatments to minimize hazard to fish; consult state fish and game agencies before applying to public waters; do not apply to muddy waters
Aqua-Kleen (2,4-D)	Union Carbide Agricultural Products Co., Inc., Research Triangle Park, North Carolina	Herbicide— 100–200 lb/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; do not use treated water for irrigation, sprays, livestock, or domestic water supplies
Aqua-Pure Algaecide Organic Copper Complex (Copper, elemental)	Malter International Corp., New Orleans, Louisiana	Algicide— 0.2–0.4 ppm	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; toxic to fish when the recommended maximum concentration at 0.4 ppm is exceeded; treat ½ of the lake or pond at one time when the rate of application is 0.4 ppm; the other ½ should be treated 7 d later to avoid oxygen depletion
Aquaquat (Diquat dibromide)	Aquacide Co., St. Paul, Minnesota	Herbicide— 8–16 oz/1,000 ft^2	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; for application only to ponds where there is little or no outflow of water and which are totally under the control of the user; do not use the treated water for animal consumption, spraying, irrigation, or domestic purposes for 14 d after treatment; do not apply to aquatic sites in Florida; to avoid fish suffocation, treat only ⅓ to ½ of the area at a time and wait 10–14 d between treatments; consult state fish and game agencies before applying to public waters
Aquashade (Acid blue and acid yellow)	Aquashade, Inc., Eldred, New York	Herbicide and algicide— 1 gal/acre, 4 ft deep	None established	Nonfood fish use; safe to use treated water for swimming and irrigation purposes
Aquathol Granular (Endothall)	Pennwalt Corp., Philadelphia, Pennsylvania	Herbicide— 13–108 lb/acre-ft	0.2 ppm in potable water	Food fish use; do not use treated water for irrigation, spraying, or domestic purposes within 7 d of treatment; do not use fish from treated water within 3 d

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
Aquathol K (Endothall)	Pennwalt Corp., Philadelphia, Pennsylvania	Herbicide—0.3–2.6 gal/acre-ft	0.2 ppm in potable water	Food fish use; do not use treated water for irrigation, spraying, or domestic purposes within 7 d of treatment; do not use fish from treated water within 3 d
Aquatic Weed Control (Diquat dibromide)	Share Corp., Milwaukee, Wisconsin	Herbicide and algicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; do not apply to muddy water; do not use treated water for human or animal consumption, swimming, spraying or irrigation within 14 d after treatment; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait at least 14 d between treatments; consult state fish and game agencies before applying to public waters
Aquatic Weed Killer (Diquat dibromide)	Venus Laboratories, Inc., Wood Dale, Illinois	Herbicide and algicide—10–20 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; delay period to use treated water for drinking—14 d, and for livestock, swimming, spraying, and irrigation—10 d; treat only ⅓ to ½ of the dense weed areas at a time and wait 10–14 d between treatments to minimize hazard to fish, consult state fish and game agencies before applying to public waters
Aquatrine (Copper, elemental)	Applied Biochemists, Inc., Mequon, Wisconsin	Algicide—0.6–1.2 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; fish or shrimp may be harvested immediately after treatment; do not use in waters containing trout if carbonate hardness of the water is less than 50 ppm; to minimize hazard to fish or shrimp, treat ⅓ to ½ of water area at a time allowing 1–2 weeks between consecutive treatments; aerate, or resume water flow 12–24 h following treatment
Aquazine (Simazine)	Ciba-Geigy Corp., Greensboro, North Carolina	Algicide and herbicide—1.7–8.5 lb/acre-ft	0.01 ppm in potable water; 12 ppm in fish	Food fish use; treated water may not be used for irrigation, spraying, livestock, or human consumption for 12 months following treatment; treated ponds may be used for swimming 4 hours after application
AV-70 Algaecide (Copper, elemental)	A & V, Inc., Pewaukee, Wisconsin	Algicide—0.75–1.5 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; do not use in water containing fish if the carbonate hardness of water is less than 50 ppm; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and treat remainder in 1–2 week intervals
AV-70 Plus Algaecide (Copper, elemental)	A & V, Inc., Pewaukee, Wisconsin	Algicide—0.62–1.25 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; water can be used immediately after treatment; do not use in water containing fish if the carbonate hardness of water is less than 50 ppm; to minimize hazard to fish, treat ⅓ to ½ of water area in a single operation and wait 10–14 d between treatments
Border-Patrol (Diquat dibromide)	Rose Chemical Products, Inc., Columbus, Ohio	Herbicide—10–40 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; delay period to use treated water for drinking—14 d, and for livestock, swimming, spraying, and irrigation—10 d; if dense weed areas are present, treat only ⅓ to ½ of the dense weed areas at a time and wait

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
				14 d between treatments to minimize hazard to fish; do not apply to muddy water; consult state fish and game agencies before applying to public waters
Clean-Flo Lake Cleanser (Aluminum sulfate, calcium sulfate, boric acid)	Clean-Flo Laboratories, Inc., Hopkins, Minnesota	Herbicide and algicide—50 lb/acre-ft	Aluminum sulfate exempted from tolerance	Food fish use; no restrictions on fishing, swimming, or irrigation; will work only in ponds and lakes that are properly aerated; is neutralized by high carbon dioxide levels in the water; for use only by applicators licensed by the company
Clean-up (Diquat dibromide)	I. Schneid, Inc., Atlanta, Georgia	Herbicide and algicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; if dense weed areas are present, treat ⅓ to ½ of the water area in a single operation and wait at least 10–14 d between treatments to minimize hazard to fish; consult state fish and game agencies before applying to public waters; do not apply to muddy water
Conkill (Diquat dibromide)	Athea Laboratories, Inc., Milwaukee, Wisconsin	Herbicide and algicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; do not apply to muddy water; for application to ponds, lakes, and drainage ditches where there is little or no outflow of water and which are totally under the control of the user; do not use the treated water for animal consumption, swimming, spraying, irrigation, or domestic purposes for 14 d after treatment; do not apply to aquatic sites in Florida; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait at least 14 d between treatments; consult state fish and game agencies before applying to public waters
Copper Sulfate (Copper sulfate)	Universal Cooperatives, Inc., Minneapolis, Minnesota	Algicide—2.75–5.5 lb/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; sectional treatments help prevent loss of fish
Copper Sulfate Granular Crystals (Copper sulfate)	Tennessee Chemical Co., Atlanta, Georgia	Algicide—0.25–2 ppm	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; trout and certain other fish species may be killed at application rates recommended on this label especially in soft or acid waters; however, fish toxicity generally decreases when the hardness of the water increases; when controlling algae in impounded waters, lakes or reservoirs (not including rice fields) and the entire body of water is to be treated, treat only ⅓ to ½ of the water area in a single operation and wait 10–14 d between treatments; consult state fish and game agencies before applying, especially to public waters; if treated water is to be used as potable water, the residual metallic copper content must not exceed 1 ppm (4 ppm copper sulfate pentahydrate)

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
Cutrine Algaecide (Copper, elemental)	Applied Biochemists, Inc., Mequon, Wisconsin	Algicide—0.75–1.5 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; water may be used immediately after treatment; do not use in water containing trout if the carbonate hardness of water is less than 50 ppm
Cutrine Granular Algaecide (Copper, elemental)	Applied Biochemists, Inc., Mequon, Wisconsin	Algicide—1 lb/430 ft²	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; water may be used immediately after treatment; never treat more than ⅓ to ½ of lake or pond at a time to avoid suffocation of fish due to lack of oxygen; do not use in water containing trout if the carbonate hardness of water is less than 50 ppm
Cutrine-Plus Algaecide (Copper, elemental)	Applied Biochemists, Inc., Mequon, Wisconsin	Algicide—0.6–1.2 gal/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; treated areas may be used for swimming or fishing immediately after treatment; do not use in water containing trout if the carbonate hardness of water is less than 50 ppm
Dichlone 50 WP (Dichlone)	FMC Corp., Agricultural Chemical Group, Philadelphia, Pennsylvania	Algicide—2.2 oz/acre-ft	None established	Nonfood fish use only; do not apply in shallow ponds that contain desirable fish; do not use in potable water; treat ⅓ of pond at a time to minimize effect on fish; re-treat in 2–3 d after decomposition of algae has occurred; consult state fish and game agencies before application
Diquatic Weed Killer (Diquat dibromide)	Lubar Co., Kansas City, Missouri	Herbicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; for application to ponds, lakes, and drainage ditches where there is little or no outflow of water and which are totally under the control of the user; do not use the treated water for animal consumption, swimming, spraying, irrigation, or domestic purposes for 14 d after treatment; when treating water areas containing fish, treat only ⅓ to ½ of the water area in a single operation; consult state fish and game agencies before applying to public waters
Formula 268 Aqua-Quat (Diquat dibromide)	The State Chemical Manufacturing Co., Cleveland, Ohio	Herbicide—10–40 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; do not apply to muddy water; for application only to ponds, lakes, and drainage ditches where there is little or no outflow of water and which are totally under the control of the user; do not use treated water for animal consumption, spraying, irrigation, or domestic purposes for 14 d after treatment; do not apply at aquatic sites in Florida; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait 10–14 d between treatments; consult state fish and game agencies before applying to public waters
Herbizole (Amitrole)	Fairmont Chemical Co., Inc., Newark, New Jersey	Herbicide—0.5–10 gal/acre	None established	Nonfood fish use only; do not apply where water will be used for irrigating, drinking, or any domestic uses; a registration standard was issued on 30 March 1984 on its use as a herbicide; EPA is initiating a special review of amitrole because it causes cancer in laboratory animals

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
Hydrothol 191 (Endothall)	Pennwalt Corp., Philadelphia, Pennsylvania	Algicide— 0.6–2.2 pt/acre-ft Herbicide— 0.7–3.4 gal/acre-ft	0.2 ppm in potable water	Food fish use; do not use treated water for livestock or domestic purposes within the following periods: up to 0.3 ppm—7 d after treatment, up to 3 ppm—14 d after application, up to 5 ppm—25 d after application; do not use fish from treated waters for food or feed within 3 d after treatment
Hydrothol 191 Granular (Endothall)	Pennwalt Corp., Philadelphia, Pennsylvania	Algicide and herbicide— 3–136 lb/acre-ft	0.2 ppm in potable water	Food fish use; do not use treated water for livestock or domestic purposes within the following periods: up to 0.3 ppm—7 d after treatment, up to 3 ppm—14 d after application, up to 5 ppm—25 d after application; do not use fish from treated waters for food or feed within 3 d after treatment
Kocide Copper Sulfate Pentahydrate Crystals (Copper sulfate)	Kocide Chemical Corp., Houston, Texas	Herbicide and algicide— 0.25–2 ppm To control tadpole shrimp— 5–10 lb/acre	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait 10–14 d between treatments; if treated water is to be used as a source of potable water, the metallic copper residual must not exceed 1 ppm (4 ppm copper sulfate pentahydrate)
Komen Aquatic Herbicide (Copper, elemental)	Kocide Chemical Corp., Houston, Texas	Herbicide— 6–16 gal/acre	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; trout and other species of fish may be killed at application rates recommended; however, fish toxicity generally decreases when the hardness of the water increases; consult state fish and game agencies before applying to public waters; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait 10–14 d between treatments
K-Tea Algaecide (Copper, elemental)	Kocide Chemical Corp., Houston, Texas	Algicide— 0.2–1 ppm/acre	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait 10–14 d between treatments; trout and other species of fish may be killed at application rates recommended; however, fish toxicity generally decreases when the hardness of the water increases; consult state fish and game agencies before applying to public waters
Mogul AG-431 (Copper, elemental)	The Mogul Corp., Chagrin Falls, Ohio	Algicide— 0.75–4.5 gal/acre	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; under conditions of heavy chara or filamentous algae growth or when treating the entire water body, treat ⅓ to ½ of the water area in a single operation; decaying vegetation depletes the oxygen content of water and will result in fish kills if an extensive area is treated at one time; treat remainder at 2 week intervals
National Chemsearch Watrol (Diquat dibromide)	National Chemsearch, Irving, Texas	Herbicide— 12–48 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; for application only to ponds, lakes, and drainage ditches where there is little or no overflow of water and which are totally under the control of the user; do not use the treated water for animal consumption, swimming, spraying, irrigation, or domestic purposes for 14 d after treatment; do not apply to aquatic

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
				sites in Florida; if dense weed areas are present, treat only ⅓ to ½ of the dense weed areas at a time and wait 14 d between treatments to minimize hazard to fish; do not apply to muddy water; consult state fish and game agencies before applying to public waters
Norkem 500 (Diquat dibromide)	Kem Manufacturing Corp., Tucker, Georgia	Herbicide and algicide—0.5–1.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; for still or slow flowing ponds, lakes, ditches, laterals, canals; is not toxic to fish or wildlife
Ortho Diquat Herbicide HA (Diquat dibromide)	Chevron Chemical Co., San Francisco, California	Algicide—0.5–1.5 ppm Herbicide—0.5–2 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; delay period before treated water can be used is 14 d for drinking and 10 d for livestock, swimming, spraying, and irrigation; do not apply to muddy water; to minimize hazard to fish, treat only ⅓ to ½ of dense weed areas and wait 10–14 d between treatment
P.D.Q. (Diquat dibromide)	I. Schneid, Inc., Atlanta, Georgia	Herbicide and algicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; if dense weed areas are present, treat ⅓ to ½ of the water area in a single operation and wait at least 10–14 d between treatments to minimize hazard to fish; consult state fish and game agencies before applying to public waters; do not apply to muddy water
Riverdale 2,4-D Granules (2,4-D)	Riverdale Chemical Co., Chicago, Illinois	Herbicide—100 lb/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; decaying vegetation in treated areas may deplete the supply of oxygen; consequent suffocation of fish may be avoided by treating ⅓ to ½ of the water area in a single operation; untreated areas may be treated after the vegetation in the originally treated areas has thoroughly decayed, approximately 2–3 months
Rodeo (Glyphosate)	Monsanto Agricultural Products Co., St. Louis, Missouri	Herbicide, annuals—1.5 pt to 3–30 gal water Herbicide, perennials—4.5–7.5 pt to 3–20 gal water	0.25 ppm in fish 0.1 ppm in potable water	Food fish use; do not apply within 0.5 mile upstream of potable water intakes
Selig's Mister Trim Number 10 (Diquat dibromide)	Selig Chemical Industries, Atlanta, Georgia	Herbicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; for application only to ponds, lakes and drainage ditches where there is little or no outflow of water and which are totally under the control of the user; do not use the treated water for animal consumption, swimming, spraying, irrigation, or domestic purposes for 14 d after treatment; do not apply to aquatic sites in Florida; when treating water areas containing fish, aquatic weed control should proceed stepwise—in bands; large areas of decaying vegetation lowers the oxygen content of the water and results in serious loss of fish; do not apply to muddy water
Sentry Yardman (Diquat dibromide)	Sentry Chemical Co., Stone Mountain, Georgia	Herbicide—0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; for application only to ponds, lakes and drainage ditches where there is little or no outflow of water and which are totally under the

Table A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
				control of the user; do not use the treated water for animal consumption, swimming, spraying, irrigation, or domestic purposes for 14 d after treatment; do not apply to muddy water; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait at least 10–14 d between treatments; consult state fish and game agencies before applying to public waters
Solricin 135 (Potassium ricinoleate)	CasChem, Inc., Bayonne, New Jersey	Algicide— 2 ppm, apply 3 times, 45 d apart Control Oscillatoria-type blue-green algae— 1.9–9.5 gal/acre-ft	Exempted from tolerance in catfish	Food fish use; apply 4 weeks before harvest to treat ponds containing off-favored fish; apply the recommended amount of product uniformly over the pond using a chemical boat; apply from shoreline outward toward the center of the pond; avoid dumping product as this can create hot spots and kill fish
Tennessee Chemical Copper Sulfate Medium Crystals (Copper sulfate)	Tennessee Chemical Co., Atlanta, Georgia	Algicide— 0.9–5.4 lb/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; if algae are visible, treat no more than ⅓ to ½ of the total pond at one time and wait 10–14 d between treatments to protect fish; trout and other fish may be killed at application rates, but fish toxicity decreases when water hardness increases
Triangle Brand Copper Sulfate (Copper sulfate)	Phelps Dodge Refining Corp., New York, New York	Algicide— 0.7–2.7 lb/acre-ft	Exempted from tolerance in meat, milk, poultry, eggs, fish, shellfish, and irrigated crops	Food fish use; restrict livestock and swimming for 12 h after treatment
Ultimate (Diquat dibromide)	Stern Chemtech Corp., Monroe, Louisiana	Herbicide— 5–20 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; delay period to use treated water for drinking—14 d, and for livestock, swimming, spraying, and irrigation—10 d; do not apply to muddy water
Vertac Weed-Rhap A-4D (2,4-D)	Vertac Chemical Corp., Jacksonville, Arkansas	Herbicide— 2.5–4.5 pt/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; do not contaminate domestic water supplies or irrigation water; to minimize hazard to fish, treat no more than ⅓ to ½ of the water area in any 1 month
Vertac Weed-Rhap LV-4D (2,4-D)	Vertac Chemical Corp., Jacksonville, Arkansas	Herbicide— 2.5–4.5 pt/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; do not contaminate domestic water supplies or irrigation water
Vertac Weed-Rhap LV-6D (2,4-D)	Vertac Chemical Corp., Jacksonville, Arkansas	Herbicide— 1.66–3 pt/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; do not contaminate domestic water supplies or irrigation water; to minimize hazard to fish, treat no more than ⅓ to ½ of the water area in any 1 month
Weedar 64 (2,4-D)	Union Carbide Agricultural Products Co., Inc., Research Triangle Park, North Carolina	Herbicide— 2.5–10 gal/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; do not use treated water for irrigation, sprays, livestock, or domestic water supplies
Weed-A-Way (Copper, elemental)	Sungro Chemicals, Inc., Los Angeles, California	Herbicide and algicide— 1 gal/2,500 ft^2 water 6 ft deep or	Exempted from tolerance in meat, milk, poultry, eggs, fish,	Food fish use; do not use treated water for irrigation or for agricultural sprays on food crops or for domestic purposes within 7 d of treatment; do not use

TABLE A-5.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
		1 gal/ft of water to maximum of 3 gal/7,500 ft^2 water less than 3 ft deep	shellfish, and irrigated crops	fish from treated water for food or feed within 3 d of treatment; to avoid a fish kill, never treat an entire lake at one time; allow at least 10–14 d between treatments to allow the chemical to dissipate and to avoid oxygen depletion; in very soft water, reduce amount by ¼, and reduce size of treatment areas to avoid a fish kill
Weed Boomer (Diquat dibromide)	Target Chemical Co., Cerritos, California	Herbicide and algicide— 0.5–1.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; do not apply to muddy water; do not use treated water for drinking purposes until 14 d after treatment; to minimize hazard to fish, treat ⅓ to ½ of the water area in a single operation and wait at least 10–14 d between treatments; consult state fish and game agencies before applying to public waters
Weed-O-Cide (Diquat dibromide)	Quality Chemical Co., North Miami, Florida	Herbicide and algicide— 0.25–2.5 ppm	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; do not apply to muddy water; do not use treated water for human or animal consumption, swimming, spraying, or irrigation within 10 d after treatment; if dense weed areas are present, treat only ⅓ to ½ of the dense weed areas at a time and wait 14 d between treatments to minimize hazard to fish; consult state fish and game agencies before applying to public waters
Weedtrine II (2,4-D)	Applied Biochemists, Inc., Mequon, Wisconsin	Herbicide— 100 lb/acre	0.1 ppm in potable water; 1 ppm in fish and shellfish	Food fish use; do not contaminate irrigation ditches or water used for domestic purposes
Weedtrine-D (Diquat dibromide)	Applied Biochemists, Inc., Mequon, Wisconsin	Algicide— 0.5–1.5 ppm Herbicide— 2.5–10 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; delay period to use treated water for drinking 14 d, and for livestock, swimming, spraying, and irrigation 10 d; do not apply to muddy water; to minimize hazard to fish, treat only ⅓ to ½ of the dense weed areas at a time and wait 10–14 d between treatments
WKC-5 (Diquat dibromide)	ABCO Inc., Irwin, Pennsylvania	Herbicide— 9.5–37 gal/acre	0.01 ppm in potable water; 0.1 ppm in fish and shellfish	Food fish use; do not apply to muddy water; for application only to ponds, lakes and drainage ditches where there is little or no outflow of water and which are totally under the control of the user; do not use the treated water for animal consumption, spraying, irrigation, or domestic purposes for 14 d after treatment; if dense weed areas are present, treat only part of dense weed areas at a time to prevent hazard to fish
Xylene (Xylene)	Union Oil Co. of California; Union Chemicals Division, Schaumburg, Illinois	Herbicide— 100 gal/acre	Exempted from tolerance when used as an aquatic herbicide applied to irrigation conveyance systems	Food fish use; for use only in programs of the U.S. Bureau of Reclamation and cooperating water user organizations; not for use in potable water; residues not to exceed 10 ppm

TABLE A-6.—Fish control agents registered or approved for aquatic or fishery uses.

Product	Sponsor	Fishery use	Tolerance	Comments
Bayluscide 5% Granular Sea Lamprey Larvicide (Bayer 73)	Mobay Chemical Corp., Kansas City, Missouri	Survey tool for sea lamprey populations—100 lb/acre	None established	Nonfood fish use only; restricted for use by certified applicators of the U.S. Fish and Wildlife Service, Fisheries and Oceans Canada, and provincial and state fish and game personnel
Bayluscide-TFM Wettable Powder Sea Lamprey Larvicide (Bayer 73, TFM)	Mobay Chemical Corp., Kansas City, Missouri	Lampricide—Mixed with TFM in proportions that result in final concentration of Bayluscide of not more than 2% of TFM by weight	None established	Nonfood fish use only; restricted for use by certified applicators of the U.S. Fish and Wildlife Service, Fisheries and Oceans Canada, and provincial and state fish and game personnel
Bayluscide 70% Wettable Powder (Bayer 73)	Mobay Chemical Corp., Kansas City, Missouri	Lampricide—Used with TFM in proportions that result in final concentration of Bayluscide of not more than 2% of TFM by weight	None established	Nonfood fish use only; restricted for use by certified applicators of the U.S. Fish and Wildlife Service, Fisheries and Oceans Canada, and provincial and state fish and game personnel
Chem-Fish Regular (Rotenone)	Tifa Limited, Millington, New Jersey	Piscicide—0.5–10 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; consult state game and fish agencies before applying
Chem-Fish Special OF (Rotenone)	Tifa Limited, Millington, New Jersey	Piscicide—0.5–10 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; consult state game and fish agencies before applying
Chem-Fish Synergized (Rotenone)	Tifa Limited, Millington, New Jersey	Piscicide—1 gal of 2.5% rotenone/6 acre-ft	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; consult state game and fish agencies before applying
Fintrol-Concentrate (Antimycin)	Aquabiotics Corp., Northbrook, Illinois	General piscicide—1–10 ppb	None established	Nonfood fish use only; fish killed with Fintrol should not be consumed by man or animals; treated water should not be consumed by man, livestock, or for crop irrigation until fingerling fish survive 48 h exposure in live cars in the treated waters; Fintrol can be used to remove scaled fish from catfish ponds
Noxfish Fish Toxicant (Rotenone)	Penick Corp., Lyndhurst, New Jersey	Piscicide—0.1–5 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; keep out of lakes, streams, or ponds except under use conditions; registered for use by, or under permit from, and after consultation with, state and federal fish and wildlife agencies

TABLE A-6.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
Nusyn-Noxfish Fish Toxicant (Rotenone)	Penick Corp., Lyndhurst, New Jersey	Piscicide—0.2–10 ppm of 2.5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment; keep out of lakes, streams, or ponds except under use conditions; registered for use by, or under permit from, and after consultation with, state and federal fish and wildlife agencies
Powdered Cube Root (Rotenone)	Penick Corp., Lyndhurst, New Jersey	Piscicide—1.36 lb of 5% rotenone/acre-ft	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only
Prentox Prenfish Toxicant (Rotenone)	Prentiss Drug and Chemical Co., Inc., New York, New York	Piscicide—0.1–5 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment; keep out of lakes, streams, or ponds except under use conditions; registered for use by, or under permit from, and after consultation with, state and federal fish and wildlife agencies
Prentox Rotenone Fish Toxicant Powder (Rotenone)	Prentiss Drug and Chemical Co., Inc., New York, New York	Piscicide—0.1–5 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment; keep out of lakes, streams, or ponds except under use conditions; registered for use by, or under permit from, and after consultation with, state and federal fish and wildlife agencies
Prentox Synprenfish Toxicant (Rotenone)	Prentiss Drug and Chemical Co., Inc., New York, New York	Piscicide—0.2–10 ppm of 2.5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment; keep out of lakes, streams, or ponds except under use conditions; registered for use by, or under permit from, and after consultation with, state and federal fish and wildlife agencies
5% Rotenone-Fish Toxicant Powder (Rotenone)	Tifa Limited, Millington, New Jersey	Piscicide—8 lb of 5% rotenone/6 acre-ft	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; consult state and federal fish and wildlife agencies before applying; dust may be irritating to eyes, skin, and mucous membranes; wear protective clothing
Rotenone Solution FK-11 (Rotenone)	Fairfield American Corp., Newark, New Jersey	Piscicide—0.5–1 ppm of 2.5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; for use only by, or under permit from, appropriate state and federal fish and wildlife agencies; do not apply to lakes, streams, or ponds except as directed on label and in accordance with appropriate state and federal fish and wildlife programs
Sea Lamprey Larvicide Lamprecid (TFM)	Hoechst Aktiengesellschaft, Frankfurt-am-Main, Germany	Lampricide—1–10 ppm, depending on the water quality	In progress	Nonfood fish use only; restricted for use by certified applicators of the U.S. Fish and Wildlife Service, Fisheries and Oceans Canada, and provincial and state fish and game personnel

TABLE A-6.—Continued.

Product	Sponsor	Fishery use	Tolerance	Comments
Security Fish-Tox-5 (Rotenone)	Woolfolk Chemical Works, Inc., Fort Valley, Georgia	Piscicide—0.5–1 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment, not for use in running waters, public lakes, or without the consent of the owner of a private pond; state game and fish agencies must be contacted before use
Security Powdered Cube (Rotenone)	Woolfolk Chemical Works, Inc., Fort Valley, Georgia	Piscicide—1.3–3 lb of 5% rotenone/acre-ft	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment, not for use in running streams, public lakes, or without the consent of the owner of a private pond
TFM Bar (TFM)	Bell Laboratories, Inc., Madison, Wisconsin	Lampricide—One bar/0.25 ft^3/s 1 ppm for 8 h at 18°C or 0.8 ppm for 10 h at 12°C	In progress	Nonfood fish use only; restricted for use by certified applicators of the U.S. Fish and Wildlife Service, Fisheries and Oceans Canada, and provincial and state fish and game personnel
Unico Rotenone Spray Powder (Rotenone)	Universal Cooperatives, Inc., Minneapolis, Minnesota	Piscicide—0.10–5.0 ppm of 5% rotenone	Exempted from tolerance when applied to growing crops; not exempted when applied to a crop at the time of or after harvest	Nonfood fish use only; restock 2–4 weeks after treatment; do not allow animals to drink the spray solution or runoff; avoid contamination of water; do not use for killing fish without first obtaining a permit from the state game and fish authorities; do not apply when weather conditions favor drift from area treated; do not apply where runoff is likely to occur; do not contaminate water by cleaning of equipment or disposal of wastes

TABLE A-7.—Conversion units.[a]

Unit	Conversion
Acre-foot	1 acre of surface area covered by 1 foot of water 43,560 cubic feet 2,718,144 pounds of water 326,000 gallons of water
Cubic foot	7.5 gallons 62.4 pounds of water 28,354.6 grams of water
Gallon	8.34 pounds of water 3,800 milliliters 3,800 grams of water
Quart	950 milliliters 950 grams of water
Pound	453.6 (454) grams 16 ounces
Ounce	28.35 grams

[a] A concentration of 1 ppm (mg/L) requires 2.7 pounds per acre-foot, 0.0038 grams per gallon, 0.0283 grams per cubic foot, or 0.0000623 pounds per cubic foot.

TABLE A-8.—Nonstandard abbreviations.

Co. = Company
Corp. = Corporation
EPA = Environmental Protection Agency
fl oz = fluid ounce
GRAS = generally recognized as safe
Inc. = Incorporated
NADA = new animal drug application
pt = pint (16 fluid ounces)
ppb = parts per billion
RPAR = rebuttable presumption against registration

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Effects of Supplemental Feeding on Growth, Production, and Feeding Habits of Striped Bass in Ponds

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Abstract.—Production of fingerling striped bass (*Morone saxatilis*) was increased by feeding fish every hour. In a 42-d study, survival and production averaged 52.8% and 167.9 kg/hectare (150 lb/acre) in ponds to which feed was delivered hourly and 34.9% and 110.6 kg/hectare (99 lb/acre) in ponds to which feed was delivered only twice a day. Types of food items selected by larvae and fingerlings in treatment and control groups were similar. Cladocerans, adult copepods, and chironomid larvae were the predominant food organisms eaten. Particles of supplemental prepared feed were found in 92% of the fish sampled from treatment ponds and in 89% of those from control ponds. The addition of supplemental feed appeared to enhance rather than to replace the natural diet.

Larval striped bass (*Morone saxatilis*) are usually reared in earthen ponds for 30–45 d and harvested when they reach about 1,540–2,220 fingerlings/kg (700–1,000/lb) (Bonn et al. 1976). Most hatchery managers rely on natural zooplankton populations to provide food for striped bass during the culture period; only three of 37 managers of striped bass hatcheries surveyed in the southeastern United States in 1980 used commercial formulated feed (Geiger and Parker 1985).

The establishment and maintenance of a preferred zooplankton forage base is essential for growth and survival of larval and fingerling striped bass in ponds (Stevens 1975). However, previous work has indicated that zooplankton populations in fertilized rearing ponds were severely depleted 2 weeks after 5-d-old fish were stocked (Geiger 1983a). Striped bass have been reported to feed continually during a 24-h period (McHugh and Heidinger 1977; Falls 1983); however most hatcheries that offer supplemental feed to striped bass do so only once or twice per day.

This study was designed to compare the effects on growth and survival of feeding striped bass either twice per day or hourly (24 times per day), and to determine feed utilization by examining contents of digestive tracts.

Methods

Automatic feeders capable of delivering formulated fry diet were installed in fertilized rearing ponds. The feeders were regulated to deliver feed hourly in selected ponds and twice per day in other ponds. The effects of frequency of feeding on growth, survival, and feeding selectivities of striped bass were evaluated during a 6-week culture period.

Pond Management

Ten 0.02-hectare (0.05-acre) striped bass rearing ponds at the Southeastern Fish Cultural Laboratory, Marion, Alabama, were filled with well water beginning on 4 April 1983. Each pond was equipped with one continuously operated airlift pump (Parker 1980) and one automatic feeder capable of delivering fry diets hourly. All ponds were initially fertilized with 567 kg/hectare (506 lb/acre) cottonseed meal, 17.8 L/hectare (1.9 gal/acre) liquid phosphoric acid (54% P_2O_5), and 15.1 L/hectare (1.6 gal/acre) liquid ammonium nitrate (32% N of NH_4NO_3). Stocking was delayed for 2 weeks due to unusually low water temperature—14–15°C (57–59°F); during each of these weeks ponds were fertilized with 284 kg/hectare (253 lb/acre) cottonseed meal, 35.6 L/hectare (3.8 gal/acre) phosphoric acid, and 30.1 L/hectare (3.2 gal/acre) ammonium nitrate. Ponds received 142 kg/hectare (127 lb/acre) cottonseed meal during the first 2 weeks after stocking, 113 kg/hectare (101 lb/acre) during the 3rd and 4th weeks, and 57 kg/hectare (51 lb/acre) during the 5th week. Liquid inorganic fertilizers were applied at the rates of 35.6 L/hectare (3.8 gal/acre) phosphoric acid and 30.1 L/hectare (3.2 gal/acre) ammonium nitrate during each of the first 2 weeks after stocking. The total amounts of fertilizers used were 1,702 kg/hectare (1,518 lb/acre) cottonseed meal, 160.2 L/hectare (17.1 gal/acre) liquid phosphoric acid, and 135.5 L/hectare (14.5 gal/acre) liquid ammonium nitrate.

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All ponds were stocked with 15-d-old fish from South Carolina at the rate of 371,000/hectare (150,000/acre) on 26 April. Fry were fed nauplii of brine shrimp (*Artemia*) ad libitum for 10 d before being stocked in the ponds. Supplemental feeding at 5.7 kg/hectare (5 lb/acre) per day was begun 11 d after stocking as recommended by Bonn et al. (1976). Fish were fed Purina Catfish Chow Starter Granule for 11 d and Silver Cup Trout Chow Number 1 granule thereafter. Fish in five control ponds were fed the daily allotment of feed twice per day at 0730 and 1500 hours, and fish in five treatment ponds were fed at hourly intervals. Fish were harvested 42 d after they were stocked in the ponds.

Water Quality

Dissolved oxygen and temperature were measured at 0700 hours daily at the surface and bottom of the water column at the drain structure. Water samples were collected twice a week from each pond and analyzed for pH, alkalinity (as $CaCO_3$), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and chlorophyll *a* by standard methods (APHA et al. 1980). Chlorophyll *a* was extracted in methanol without grinding as recommended by Holm-Hansen and Riemann (1978). Emergency aeration was provided by 0.22-kW surface agitators whenever dissolved oxygen concentration fell below 3 mg/L.

Sampling and Analysis

Zooplankton samples were collected twice weekly from each pond with a flexible-impeller pump, using the procedure described by Geiger (1983b). Organisms were collected in a 10-L (2.6-gal) water sample from the deep end of the pond. The sample was filtered through an 80-μm Wisconsin plankton net, and the organisms collected were preserved in 4% buffered formalin–sucrose. Organisms were counted, identified to the lowest possible taxon (Ward and Whipple 1959; Pennak 1978), and expressed as numbers of organisms per liter.

Striped bass were collected weekly with a push net beginning 6 d after stocking. Ten fish were usually collected from each pond and preserved in chilled 70% ethyl alcohol. Each fish was measured (standard length, mm), weighed (mg), numbered, and frozen at −20°C (−4°F) for later analysis.

Frozen fish were thawed and the entire digestive tracts were removed, blotted, and weighed to the nearest 0.01 mg. Digestive tract contents were then flushed into individually marked containers and preserved in 4% buffered formalin–sucrose for later analysis. The empty digestive tracts were weighed to the nearest 0.01 mg to give an estimated weight of the contents. Digestive tract contents were flushed into a Sedgewick-Rafter counting chamber and macroinvertebrates were enumerated, identified, and removed under a dissecting microscope at 12× or 25× magnification. The remaining food items were counted under a microscope at 50× or 100× magnification and identified to the lowest recognizable taxon. Each digestive tract was examined for the presence or absence of organic matter believed to be the remains of supplemental feed.

The digestive tracts of fish collected during the first week were not weighed. The fish were placed in a Sedgewick-Rafter counting chamber and entire digestive tracts were removed under a dissecting microscope. Contents were enumerated and identified as previously described.

Biological data were $\log_{10}$ transformed before statistical analysis to reduce the possible heterogeneity of variances. Data were analyzed by analysis of variance and Duncan's multiple-range test. Statements of statistical significance refer to $P < 0.05$.

Results and Discussion

Fish Production

Fish production and survival after 42 d were greater in treatment ponds in which fish were fed hourly than in control ponds in which fish were fed only twice daily (Table 1). Survival averaged 52.8% and production 167.9 kg/hectare (150 lb/acre) in treatment ponds compared with 34.9% and 110.6 kg/hectare (99 lb/acre) in control ponds. Weekly fish weight and length measurements were generally similar among fish sampled from treatment and control ponds (Table 2). Fish sampled from the treatment ponds were significantly heavier than those sampled from control ponds in week 4. Fish in all ponds remained in the deepest part of the pond near the drain structure during the first

TABLE 1.—Summary of striped bass harvesting data after 42 d of culture in five 0.02-hectare (0.05-acre) ponds per category.

Pond category	Density		Weight (fish/kg)	Survival (%)
	Number/hectare	Kg/hectare		
Treatment	229,700	149.8	1,534	61.3
(hourly feeding)	211,000	202.8	1,040	56.3
	330,100	306.3	1,078	88.0
	114,600	100.0	1,146	30.6
	104,150	80.7	1,290	27.8
Mean	197,910	167.9	1,218	52.8
Control	80,800	91.5	891	21.5
(twice-daily feeding)	236,050	195.0	1,210	62.9
	102,650	54.4	1,907	27.4
	106,850	91.2	1,173	28.5
	127,350	121.1	1,052	34.0
Mean	130,740	110.6	1,247	34.9

2 weeks of the culture period. After the 3rd week, those in the control ponds were in schools throughout the ponds, whereas those in the treatment ponds were usually beneath the automatic feeders. Fish in all ponds were difficult to sample after the 2nd week; the differences in size between treatment and control fish were probably due to our inability to collect the larger fish in the control ponds because they were traveling in scattered schools throughout each pond. Lengths and weights of about 20 fish per pond were recorded at harvest and no significant differences in size between treatment and control fish were found (Table 2).

TABLE 2.—Mean length and weight of striped bass sampled weekly from 0.02-hectare (0.05-acre) rearing ponds.

Week of study	Age of fish (d)	Pond category[a]	Number of fish	Length (mm)	Weight (mg)
				Mean±SD[b]	Mean±SD[b]
1	21	T	38	9.5±0.7*	13.8±3.5
		C	36	9.9±0.7	15.5±5.0
2	28	T	48	14.2±1.3	50.7±14.4
		C	48	13.9±1.5	48.1±21.1
3	35	T	50	18.8±2.9	135.1±71.5
		C	50	19.3±3.1	139.3±72.2
4	43	T	43	22.4±4.6	235.1±140.4*
		C	50	21.0±4.0	182.5±101.1
5	50	T	50	27.4±6.0	482.4±378.6
		C	45	27.0±4.1	390.5±205.6
6	57	T	99	36.2±5.7	953.0±515.0
		C	99	36.1±7.4	963.0±802.0

[a] T = treatment (hourly feeding); C = control (twice-daily feeding).

[b] Asterisk (*) indicates means for treatment and control groups were significantly different ($P < 0.05$) from each other within weekly samples.

TABLE 3.—Mean values for water quality variables measured weekly in 0.02-hectare (0.05-acre) striped bass rearing ponds.

Variable	Pond category[a]	Number of samples	Mean±SD[b]
pH	T	65	8.2±0.6
	C	65	8.1±0.6
Alkalinity	T	65	87.4±14.1
(mg/L as $CaCO_3$)	C	65	90.8±13.9
NH_3-N (mg/L)	T	65	0.1±0.1
	C	65	0.1±0.1
NO_2-N (mg/L)	T	65	0.02±0.04*
	C	65	0.01±0.02
NO_3-N (mg/L)	T	65	0.5±0.4
	C	65	0.4±0.4
Chlorophyll *a*	T	60	32.4±32.8*
(μg/L)	C	60	20.6±23.8
PO_4-P (mg/L)	T	55	0.5±0.4
	C	54	0.4±0.3
Dissolved oxygen (mg/L)			
Surface	T	218	6.9±2.4*
	C	218	6.5±2.3
Bottom	T	218	6.8±2.4*
	C	218	6.3±2.3

[a] T = treatment (hourly feeding); C = control (twice-daily feedings).

[b] Asterisk (*) indicates means for treatment and control groups were significantly different ($P < 0.05$) within weekly samples.

Water Quality

Treatment and control ponds differed significantly in dissolved oxygen and nitrite-nitrogen and chlorophyll *a* (Table 3). Dissolved oxygen concentrations differed significantly between treatment and control ponds at each depth sampled during weeks 1, 2, 4, and 6, but rarely fell below 3 mg/L in any ponds during the study. Water temperatures recorded at the surface and bottom of each pond were never significantly different between and within treatment and control ponds; overall mean ± SD water temperatures were 21.7 ± 3.0°C (71 ± 5°F). Alkalinity and pH were significantly higher in treatment ponds than in control ponds during week 2. Nitrate concentrations were significantly higher in treatment ponds than in control ponds during week 4. Chlorophyll *a* was consistently higher in treatment ponds than in control ponds during all post-stocking weeks.

Zooplankton

Ponds in which fish were fed hourly generally produced more rotifers and cladocerans than did

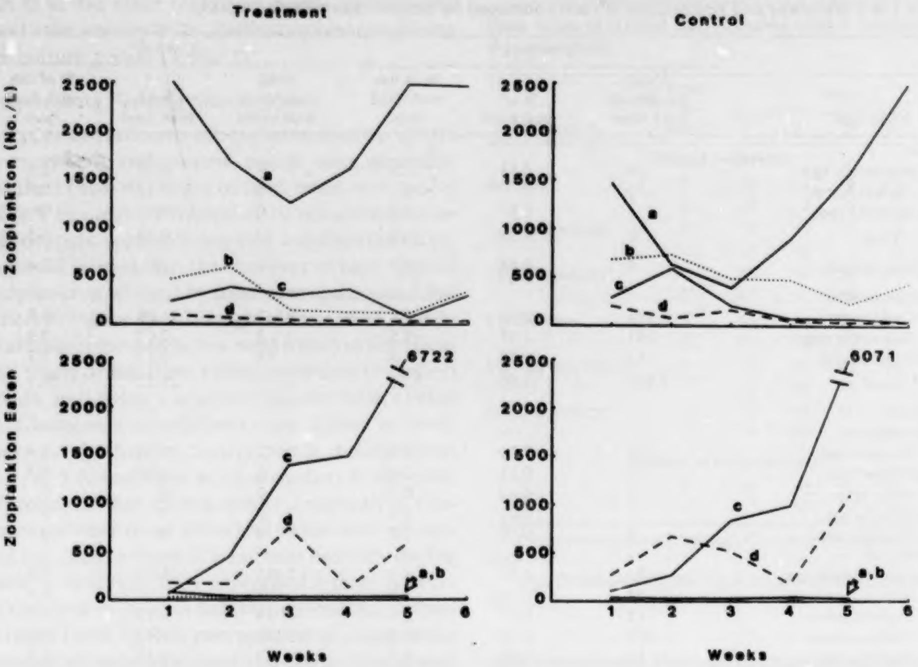

FIGURE 1.—Mean numbers of rotifers (a), copepod nauplii (b), cladocerans (c), and copepod adults (d) sampled twice a week from treatment and control ponds (upper panels) and total numbers of these organisms eaten by striped bass in these ponds (lower panels). Fish were supplementally fed every hour (24 times per day) in treatment ponds and twice daily in control ponds.

control ponds (Figure 1). Rotifer populations remained high in all ponds throughout the study, but were lowest in week 3. The numbers of copepod nauplii and adults (*Cyclops* spp. and *Diaptomus* spp.) were consistently higher in control ponds than in treatment ponds and peaked in week 2. Cladoceran populations (primarily *Moina* spp., *Daphnia pulex,* and *Simocephalus* spp.) peaked in week 2 and populations of *Ceriodaphnia, Chydorus,* and *Bosmina* appeared thereafter, in that order.

Treatment ponds, which had denser phytoplankton blooms (judged by chlorophyll-*a* levels) than control ponds, generally supported higher rotifer and cladoceran populations (Figure 1). The rotifers *Brachionus* and *Euchlanis* are generally considered to be indicators of a eutrophic habitat (U.S. Environmental Protection Agency 1979). These genera of rotifers were more numerous in the zooplankton samples from treatment ponds than from control ponds. When the phytoplankton in the control ponds was depleted, the copepods (*Cyclops* adults and nauplii) became the predominant zooplankters. They have an advantage over rotifers and cladocerans both in being cannibalistic and in being able to feed on organisms of a greater size range (Allan 1976); cyclopoid copepods (*Cyclops* spp.) have also been reported to feed almost exclusively on organic debris under certain circumstances (Pennak 1978).

Peak populations of preferred zooplankton such as copepods and cladocerans were found 4 to 5 weeks into the culture period, as compared with only 2 to 3 weeks in another study (Geiger et al. 1985) in which ponds were fertilized but the fish were not supplementally fed. The higher phytoplankton populations in the treatment ponds generally supported greater numbers of filter-feeding zooplankters. The more eutrophic conditions in the treatment ponds may have been a result of the continued enrichment of the water with feed.

One treatment pond, however, supported fewer rotifers and crustaceans than did the other treatment ponds—probably because it supported an extremely dense population of fairy shrimp (Anostraca). This pond produced fewer and smaller

TABLE 4.—Numbers and percentages of food consumed by striped bass sampled weekly.

Food item	Treatment[a]			Control[b]		
	Total number of food items	% of total food	% of fish with food item	Total number of food items	% of total food	% of fish with food item
Rotifera						
Brachionus spp.	69	0.53	10.8	9	0.07	2.6
Euchlanis spp.	0	0	0	15	0.12	0.9
Keratella spp.	1	0.01	0.5	0	0	0
Total	70	0.54	10.8	24	0.19	3.5
Copepoda (nauplii)	5	0.04	2.4	1	0.01	0.4
Copepoda (adults)						
Cyclops spp.	1,294	10.00	66.2	2,562	21.08	75.8
Diaptomus spp.	351	2.71	7.5	3	0.02	0.9
Unidentified spp.	12	0.09	1.9	14	0.12	3.1
Total	1,657	12.80	72.3	2,579	21.22	76.7
Cladocera						
Bosminidae						
Bosmina spp.	976	7.54	9.9	2	0.02	0.4
Chydoridae spp.	16	0.12	2.4	2	0.02	0.4
Alona spp.	3	0.02	0.5	0	0	0
Alonella spp.	1	0.01	0.5	0	0	0
Chydorus spp.	13	0.10	2.8	0	0	0
Daphnidae						
Ceriodaphnia spp.	631	4.99	7.5	3,481	28.65	7.5
Daphnia ambigua	24	0.19	3.3	108	0.90	4.9
Daphnia laevis	15	0.12	2.4	16	0.13	4.4
Daphnia pulex	818	6.32	10.8	179	1.47	9.7
Daphnia spp.	10	0.08	3.3	15	0.12	2.6
Moina spp.	182	1.40	8.9	31	0.26	4.9
Scapholeberis spp.	247	1.90	0.9	104	0.86	1.3
Simocephalus spp.	3,351	25.90	32.9	763	6.28	29.5
Macrothricidae						
Macrothrix rosea	326	2.52	4.2	261	2.15	2.2
Macrothrix spp.	1	0.01	0.5	116	0.96	6.6
Sididae						
Diaphanosoma spp.	64	0.49	3.2	0	0	0
Unidentified spp.	3,335	25.77	75.6	3,083	25.37	74.9
Total	10,013	77.39	81.7	8,163	67.19	78.4
Diptera						
Chironomidae						
Pupae	86	0.67	18.7	112	0.92	25.1
Larvae	1,068	8.25	61.5	1,194	9.83	66.9
Adults	0	0	0	14	0.12	2.6
Tanypodinae adults	1	0.01	0.5	0	0	0
Culicidae						
Chaoborinae pupae	1	0.01	0.5	0	0	0
Chaoborinae larvae	1	0.01	0.5	1	0.01	0.4
Heleidae (larvae)	3	0.02	0.5	11	0.09	2.6
Unidentified pupae	2	0.02	0.9	5	0.05	2.2
Total	1,162	8.98	63.9	1,337	11.02	70.0
Hemiptera						
Corixidae	9	0.07	0.9	16	0.12	0.4
Notonectidae	8	0.06	1.4	2	0.02	1.3
Total	17	0.13	2.3	18	0.14	1.8
Ostracoda	12	0.09	3.3	6	0.05	2.2
Eubranchiopoda	3	0.02	0.9	0	0	0
Nematoda	0	0	0	6	0.05	2.6
Organic matter[c]			92.2			89.0

[a] Of 219 fish sampled, 213 contained food.

[b] Of 230 fish sampled, 227 contained food.

[c] Organic matter is defined as particles recognizable as feed.

fish than did other treatment ponds, presumably due to the scarcity of available zooplankton during the culture period (Table 1).

Striped Bass Feeding Selectivities

Types and amounts of prey eaten by striped bass in treatment and control ponds were generally similar (Table 4). Types of food items selected by all fish in order of frequency of occurrence were cladocerans, copepods (adults), and dipterans (chironomid larvae), but the numbers of each type of zooplankton selected by treatment and control fish differed (Figure 1). Although rotifers were readily available in the ponds, few were found in the digestive tracts of fish from either treatment or control ponds, indicating a selection against them (Table 5). Cladoceran populations were higher in treatment ponds than in control ponds during weeks 3, 4, and 6, and were more abundant in the diets of treatment fish during weeks 2 through 5. Cladocerans were more abundant in the diets of control fish than in those of treatment fish only during week 1; however, they composed a large portion of the food of control fish throughout the culture period (Table 5). The composition of cladocerans differed between treatment and control ponds and also between the diets of fish in ponds of each category. Cladocerans selected by fish, in order of total numbers, were *Simocephalus, Bosmina, Daphnia, Ceriodaphnia, Macrothrix, Scapholeberis,* and *Moina* in the treatment ponds; and *Ceriodaphnia, Simocephalus, Macrothrix, Daphnia,* and *Scapholeberis* in the control ponds (Table 4).

Copepods were more abundant in control ponds than in treatment ponds during all weeks of the study (Figure 1) and fish in control ponds ate more adult copepods during weeks 1 and 3–5 than did fish in treatment ponds (Table 5). The higher numbers of copepod adults in the digestive tracts of treatment fish during week 2 were calanoids (*Diaptomus* spp.); these copepods are primarily filter feeders and were possibly more abundant in treatment than in control ponds because the phytoplankton populations were denser in the treatment ponds.

Dipterans (primarily chironomid larvae and pupae) first appeared in the diets of striped bass during week 2. The numbers of dipterans eaten by fish in treatment and control ponds were generally similar on a week-by-week basis, although fish in the control ponds ate slightly higher numbers of chironomid larvae than did fish in treatment ponds (Table 5). Eubranchiopods, nematodes, ostracods, and hemipterans also infrequently appeared in the diets of treatment and control fish during weeks 2 to 5 (Table 4).

TABLE 5.—Percent frequency of occurrence of selected food items in striped bass sampled weekly beginning 6 d poststocking.

Food item	Pond category[a]	Week 1	Week 2	Week 3	Week 4	Week 5
		Percent occurrence				
Rotifers	T	26	15	8	3	4
	C	6	2	6	4	0
Copepod nauplii	T	3	9	0	0	0
	C	0	0	0	0	2
Copepod adults	T	71	76	76	42	86
	C	97	51	88	62	91
Cladocerans	T	48	74	92	88	98
	C	61	68	80	86	91
Chironomid larvae	T	0	37	82	94	84
	C	0	48	88	98	82
Organic matter[b]	T	0	100	88	94	84
	C	0	96	82	84	98
		Number of fish examined				
	T	38	48	50	33	50
	C	37	48	50	50	44

[a] T = treatment (hourly feeding); C = control (twice-daily feeding).

[b] Organic matter is defined as food particles recognizable as feed.

The remains of supplemental feed were found in 92% of the fish from the treatment ponds and in 89% of those from control ponds. The high percentage of feed found in the digestive tracts of control fish is probably due to the fact that fish were always sampled about 2–3 h after the morning feeding period. Most food items in the digestive tracts were well digested and the high incidence of unrecognizable cladocerans (Table 4) may be attributed to the distortion these zooplankters undergo during digestion. Only one fish (sampled during week 5 from a control pond) was observed to have the remains of another striped bass in its digestive tract.

Despite the abundance of supplemental feed provided in treatment ponds, the fish continued to feed heavily on natural prey. Few fish from any pond had only the remains of supplemental feed in their digestive tracts; most had remains of cladocerans, copepods, or chironomids in addition to small amounts of supplemental feed.

The selectivity of food organisms depends to some degree on the size of the fish and the relative proportions of the food organisms available (Humphries and Cumming 1972). This selectivity pattern was exemplified by the consumption of early instars of copepods and cladocerans during the first 2 weeks, followed by small instars of dipterans in weeks 2 and 3. Adult instars of cladocerans and copepods and large instars of dipterans were eaten by fish during weeks 3–5. Hemipterans, primarily Corixidae, appeared in the diets of treatment and control fish in weeks 4 and 5. This selection pattern of orders of invertebrates by striped bass larvae and fingerlings (Copepoda, Cladocera, Diptera, Hemiptera) also has been reported by other investigators (Sandoz and Johnston 1966; Doroshev 1970; Humphries and Cumming 1972; Harrell et al. 1977; Falls 1983).

The selection against smaller prey (rotifers and copepod nauplii) in this study was due primarily to the age and size of the fish at stocking. The delay of stocking in this study necessitated that the striped bass be maintained on brine shrimp nauplii until 15 d of age. The fish were 21 d old at the first sampling period, and by then were able to feed on larger food particles.

In summary, striped bass fingerling survival was 18% greater and production was increased by 57 kg/hectare (51 lb/acre) when fish were supplementally fed a formulated feed 24 times per day, as compared to control fish which were fed twice per day. The increased frequency of supplemental feeding appeared to increase not only fish production and survival, but helped to maintain zooplankton populations in the ponds for longer periods during the culture period. Fish that were fed 24 h per day were readily trained to accept the feed, in addition to available prey organisms.

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Feeding Rate, Water Quality, and Channel Catfish Production in Ponds

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Abstract.—Channel catfish (*Ictalurus punctatus*) were stocked in ponds at 1,200, 4,300, 8,600, 17,300, 26,000, and 34,600 fish per hectare (486, 1,740, 3,480, 7,000, 10,520, and 14,000 fish per acre), and maximum daily feeding rates of 0, 28, 56, 84, 112, 168, and 224 kg/hectare (0. 25, 50, 75, 100, 150, and 200 lb/acre), respectively, were established. Aeration was applied at 6.1 kW/hectare (3.3 horsepower/acre) when dissolved oxygen (DO) concentrations were expected to fall below 2 mg/L. Strong correlations were obtained between feeding rates (X) and concentrations (Y) of DO at dawn, chlorophyll *a*, chemical oxygen demand, carbon dioxide, and total ammonia nitrogen. The correlation for DO was negative. Net fish production was correlated with feeding rates up to a maximum daily feeding rate of 112 kg/hectare (100 lb/acre). Feed conversion ratios were essentially constant up to a maximum feeding rate of 112 kg/hectare (100 lb/acre). At higher feeding rates, feed conversion ratios increased and fish production declined. Little aeration was required at feeding rates of 56 kg/hectare (50 lb/acre) or less; aeration was applied almost constantly at night in ponds with maximum daily feeding rates of 112 kg/hectare (100 lb/acre) and above.

Concentrations of chlorophyll *a*, total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), carbon dioxide (CO_2), and chemical oxygen demand (COD), and the likelihood of insufficient levels of dissolved oxygen (DO) at night or in the early morning increase with increasing feeding rates in channel catfish (*Ictalurus punctatus*) ponds (Boyd et al. 1979; Tucker et al. 1979; Boyd 1982). Furthermore, feed conversion ratios (feed applied ÷ net fish production) increase with feeding rate (Tucker et al. 1979). These studies considered only two or three feeding rates, and the relationships between feeding rates and either water quality or fish production have not been defined precisely.

The present research considers the influence of seven feeding rates upon water quality and channel catfish production.

Methods

Ponds at the Auburn University Fisheries Research Unit which were 0.04 hectare (0.1 acre) in area with average depths of 1 m (3.3 ft) were used in this research. Water levels were maintained (without overflow) by additions of water from a reservoir. This water was low in nutrients and organic matter: total alkalinity and hardness, 8 to 12 mg/L as $CaCO_3$; total nitrogen, <0.5 mg/L; total phosphorus, <0.1 mg/L; chemical oxygen demand, <20 mg/L.

Channel catfish weighing an average of 18 g (0.6 oz) each were stocked at rates ranging from 1,200 to 34,600 fish per hectare (486–14,002 fish per acre) on 15 February 1983 (Table 1). Fish were fed at 3% of body weight per day, adjusted every 2 weeks for growth by assuming a feed conversion ratio of 1.5. The feed consisted of floating pellets containing 32% crude protein. Maximum feeding rates (Table 1), ranging from 0 to 224 kg/hectare/d (0–200 lb/acre/d) were reached in mid-August; rates were then held constant until fish harvest began on 1 October 1983.

Each pond was fitted with a 0.25-kW (0.33-horsepower) surface aerator (vertical turbine type)—6.1 kW of aeration per hectare (3.3 horsepower/acre). The nighttime DO model described by Boyd et al. (1978) was used to determine the ponds in which DO was likely to fall below 2 mg/L on a particular night. Aeration was initiated in an individual pond just before the DO concentration was expected to reach 2 mg/L. It was sometimes necessary to aerate

TABLE 1.—Stocking rates and maximum daily feeding rates for channel catfish ponds.

Stocking rate (fish/hectare)	Maximum feeding rate (kg/hectare/d)
1,200	0
4,300	28
8,600	56
13,000	84
17,300	112
26,000	168
34,600	224

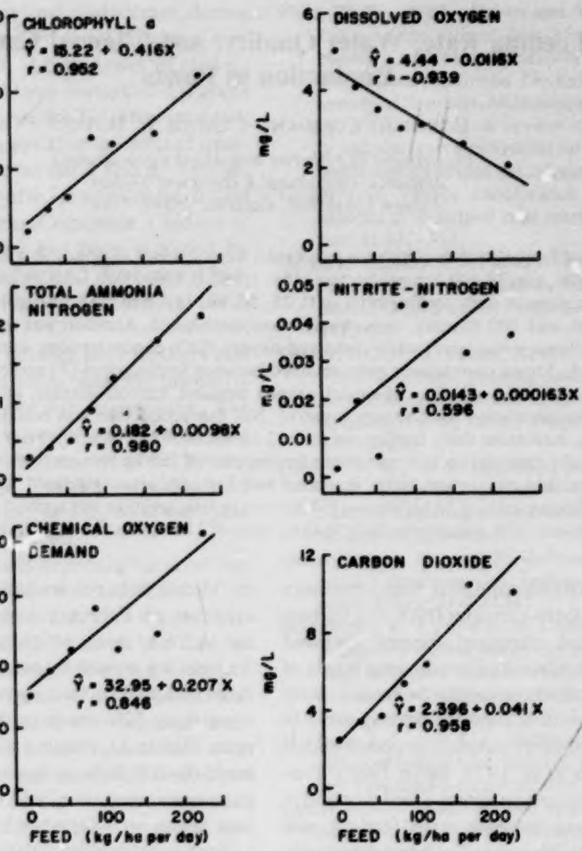

FIGURE 1.—Average concentrations of water quality variables in channel catfish ponds with different maximum daily feeding rates.

ponds during daylight hours. The number of hours of aeration for each pond was recorded.

Measurements of DO at a depth of 5 cm (2 in) were made with a polarographic DO meter daily at dawn and dusk between May and October. Water samples were collected monthly with a 90-cm (35-in) column sampler (Boyd 1979) between May and October. Samples were analyzed according to APHA et al. (1980) for NO_2-N (diazotization), TAN (phenate method), CO_2 (sodium carbonate titration), chlorophyll *a* (acetone extraction and spectroscopy), and COD (sulfuric acid–dichromate oxidation).

Results and Discussion

Concentrations of chlorophyll *a*, TAN, NO_2-N, COD, and CO_2 increased and concentrations of DO at dawn decreased in all ponds receiving feed as the growing season progressed; similar findings were presented by Boyd et al. (1979), Tucker et al. (1979), and Hollerman and Boyd (1980). Minimum concentrations of DO and maximum concentrations of other variables occurred in August and September (Table 2). Ponds with higher feeding rates (168 and 224 kg/hectare/d) (150 and 200 lb/acre/d) had dense phytoplankton blooms and high COD values. Concentrations of DO at dawn were often near or at 0 mg/L, even though aerators were running, and CO_2 values were frequently above 10 mg/L. The combination of low DO and high CO_2 is particularly harmful to fish (Boyd 1982). Concentrations of TAN were frequently 2–4 mg/L in ponds receiving large amounts of feed. Un-ionized ammonia nitrogen (NH_3-N) and am-

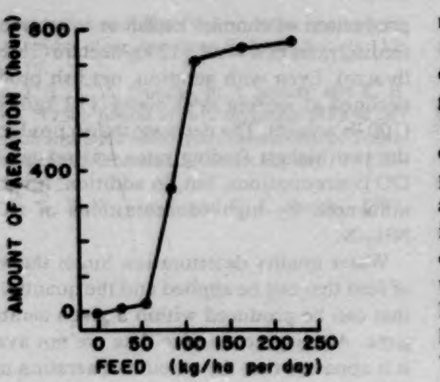

FIGURE 2.—Total hours of aeration (6.1 kW/hectare) applied to channel catfish ponds with different maximum daily feeding rates.

monium nitrogen (NH_4-N) comprise TAN. The toxic form, NH_3-N, exists in a pH- and temperature-dependent equilibrium with NH_4-N; the proportion of NH_3-N increases as temperature and pH rise. Because pH may increase markedly in catfish ponds during daylight hours (Boyd 1982), the potential for high NH_3-N concentrations exists when TAN values are high. During afternoons, pH values were frequently between 8.5 and 9.5. According to Boyd et al. (1979), at 30°C (86°F) and a pH of 9, 44.8% of the TAN is in un-ionized form (NH_3-N). Thus, NH_3-N concentrations of 0.9–1.8 mg/L were not uncommon during afternoons but, during nights and mornings, pH was lower and NH_3-N values were less. The 24-h LC50 of NH_3-N to channel catfish has been reported to range between 1.39 and 2.36 mg/L (Robinette 1976; Tomasso et al. 1980), and much lower concentrations apparently have adverse effects on growth (Robinette 1976; Colt and Tchobanoglous 1978). Tucker et al. (1984) indicated that the highest concentrations of TAN in ponds can be expected in the fall after water temperatures decline and phytoplankton blooms diminish. Ponds used in the present research were drained before the critical period for TAN. Concentrations of NO_2-N were never high enough to harm fish. However, NO_2-N concentrations are known to reach toxic levels in channel catfish ponds (Schwedler and Tucker 1983).

Data for all sampling dates were averaged by treatment, and regression equations between feeding rates (X) and water quality variables (Y) were calculated (Figure 1). Strong correlations were obtained between feeding rate and chlorophyll *a*, TAN, DO, COD, and CO_2. The correlation between feeding rate and NO_2-N was weak.

Aeration no doubt influenced the relationship between feeding rates and DO concentrations at dawn, but DO concentrations still were related strongly to feeding rate (Figure 1). The total number of hours of aeration increased with feeding rate (Figure 2), but the relationship was not linear. Ponds with low feeding rates (0, 28, and 56 kg/hectare/d) (0, 25, and 50 lb/acre/d) required little aeration; the increase in aeration between feeding rate treatments of 56 and 112 kg/hectare/d (50 and 100 lb/acre/d) was linear. Between 112 and 224 kg/hectare/d (100 and 200 lb/acre/d), there was little increase in aeration, for during August and September aerators were operated almost con-

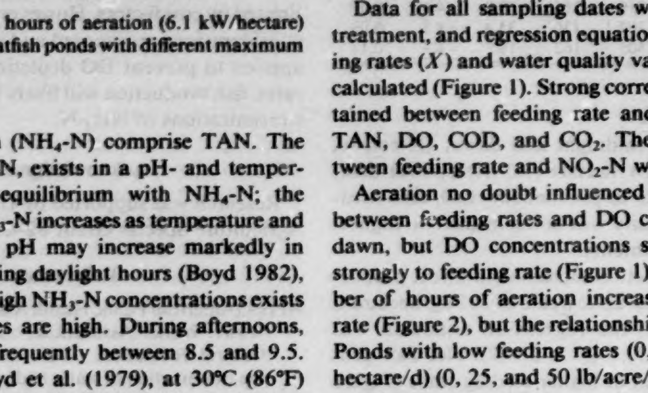

FIGURE 3.—Average net fish production and feed conversion ratios for channel catfish fed at different maximum daily rates.

TABLE 2.—Minimum concentrations of dissolved oxygen (DO) and maximum concentrations of chlorophyll *a* (Chl), chemical oxygen demand (COD), carbon dioxide (CO_2), total ammonia nitrogen (TAN), and nitrite-nitrogen (NO_2-N) in channel catfish ponds with different feeding rates.

Maximum feeding rate (kg/hectare/d)	Water quality variables					
	DO (mg/L)	Chl (μg/L)	COD (mg/L)	CO_2 (mg/L)	TAN (mg/L)	NO_2-N (mg/L)
0	5.1	50	50	8.9	0.9	0.01
28	4.2	95	82	10.2	1.0	0.11
56	1.9	105	99	7.5	2.6	0.01
84	1.0	192	178	13.4	4.2	0.10
112	0.5	310	265	13.0	4.1	0.09
168	0.0	205	150	35.4	4.5	0.09
224	0.0	405	165	18.2	4.7	0.11

stantly between midnight and dawn. Of course, aeration at 6.1 kW/hectare (3.3 horsepower/acre) was not adequate to prevent low DO, and occasional fish mortality was noted in the two highest feeding rate treatments.

Net fish production increased in direct proportion to feeding rate up to a rate of 112 kg/hectare/d (100 lb/acre/d); the regression equation was $\hat{Y} = 161.6 + 51.77X$ ($r = 0.999$). Net fish production decreased at higher feeding rates (Figure 3). Feed conversion ratios were essentially constant between feeding rates of 28 and 112 kg/hectare/d (25 and 100 lb/acre/d), but they increased drastically at higher feeding rates (Figure 3). Because fish did not grow as well as expected at feeding rates above 112 kg/hectare/d (100 lb/acre/d), they did not consume all of the feed applied. No estimate of actual feed consumption was obtained. This practice obviously inflated feed conversion values for feeding rates above 112 kg/hectare/d (100 lb/acre/d).

The findings reported above provide a more precise assessment of the relationships among feeding rates, water quality, and net production of channel catfish than previously available. There are strong correlations between feeding rates and phytoplankton abundance (chlorophyll *a*), COD, DO, CO_2, and TAN. Hence, as feeding rates increase, water quality deteriorates proportionally. Little aeration is required at feeding rates up to 56 kg/hectare/d (50 lb/acre/d). Aeration at 6.1 kW/hectare (3.3 horsepower/acre) when DO concentrations were low was adequate to sustain a constant feed conversion ratio and a linear increase in net production of channel catfish at maximum daily feeding rates of 84 and 112 kg/hectare (75 and 100 lb/acre). Even with aeration, net fish production declined at feeding rates above 112 kg/hectare/d (100 lb/acre/d). The decrease in fish production at the two highest feeding rates was related to low DO concentrations, but, in addition, it was likely influenced by high concentrations of CO_2 and NH_3-N.

Water quality deterioration limits the amount of feed that can be applied and the quantity of fish that can be produced within a given aeration regime. Although economic data are not available, it is apparent that the amount of aeration must be limited by cost factors. However, even if economic considerations are ignored and enough aeration is applied to prevent DO depletion at high feeding rates, fish production will likely be limited by large concentrations of NH_3-N.

Acknowledgment

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Effects of Rearing Density on Growth and Survival of Lake Trout

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Abstract.—Lake trout (*Salvelinus namaycush*) were reared at density indices (allowable density in pounds/cubic foot divided by fish length in inches) of 0.25, 0.50, 1.0, and 2.0 in cages suspended in circular tanks. Water flow rates were such that dissolved oxygen remained above 8 ppm within all the cages. Thus treatment effects were attributed to density and not influenced by water quality. Growth and condition were not affected by density, but survival was lower at the higher density than at the three lower densities. We conclude that lake trout may be successfully reared at a density index of at least 1.0 if the water requirements for respiration and waste dilution are met.

Tunison (1945) presented recommended densities for trout in troughs and ponds and demonstrated that acceptable densities increase with increasing fish size. Haskell (1955) related permissible fish densities to feeding rate, recognizing that carrying capacity was a function of oxygen consumption and metabolite production, and that these parameters were proportional to the amount of food metabolized by the fish. Willoughby (1968) presented a formula that related oxygen consumption to the amount of food fed. These papers all express density as a function of oxygen consumption and do not address the spatial requirements of cultured fish.

Buss et al. (1970) and Piper (1975) described the independence of water requirements and spatial requirements of cultured fish. The water requirements of salmonids are well known (Liao 1971; Piper 1975; Westers and Pratt 1977; Soderberg, in press), but their spatial requirements are rather poorly defined.

Piper (1975) related recommended fish density to fish length in a useful term called density index. The density index is simply the allowable density in pounds/cubic foot divided by the fish length in inches. A density index of 0.5 was recommended as a safe value for use in hatcheries (Piper 1975). Thus 8-in trout could safely be reared at a density of 4 lb/ft^3 as long as their water requirements for respiration and metabolite dilution were met. Trout have been reared at densities considerably greater than is common at most hatcheries. Buss et al. (1970) reported successfully rearing rainbow trout (*Salmo gairdneri*) at 34 lb/ft^3 in hatchery jars. Clary (1979) found that trout were not adversely affected when reared at a density index of 0.8.

The purpose of the present study was to investigate the effects of density, independent of water flow, on lake trout (*Salvelinus namaycush*) in intensive culture.

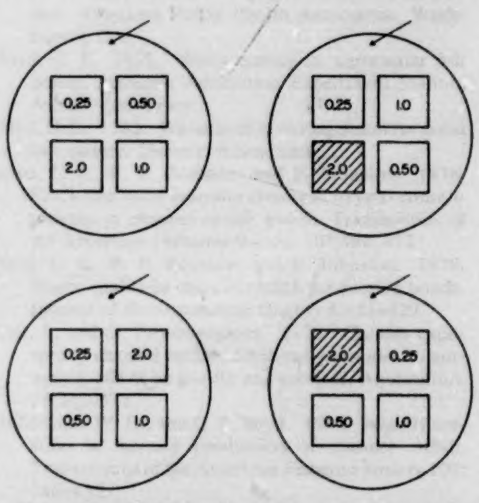

FIGURE 1.—Positions of experimental units, relative to water inflow, and location of replicates experiencing disease epizootics in lake trout density study. The arrow indicates location and direction of water inflow. The number in the box is the density index of that replicate, and the shaded area indicates location of a disease epizootic.

TABLE 1.—Growth and mortality of lake trout reared at different densities and constant water quality for 97 d. Each value is the mean of four replicates. Values within a column followed by the same letter are not significantly different ($P > 0.05$).

Density index	Density (lb/ft^3)		Mean length (in)		Mean weight (oz)		Mean condition factor[a]		Mortality (%)
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
0.25	1.7	1.9	6.89	7.83 z	1.66	2.08 z	3,169	2,690 z	1.00 z
0.50	3.4	3.8	6.89	7.64 z	1.66	2.22 z	3,169	3,092 z	2.25 z
1.0	6.9	7.7	6.89	7.64 z	1.66	1.98 z	3,169	2,774 z	3.25 z
2.0	13.8	15.3	6.89	7.56 z	1.66	1.83 z	3,169	2,673 z	25.25 y

[a] Condition factor = $10^7 \times$ weight (lb)/length $(in)^3$.

Methods

Lake trout, 6.89 in in length, were stocked in 4.41 ft^3 cages constructed from ¼-in plastic screen, suspended in 6-ft-diameter circular fiberglass rearing tanks. Each of the four rearing tanks contained four cages stocked at density indices of 0.25, 0.50, 1.0, and 2.0 (Piper 1975). Initial stocking densities were 1.7, 3.4, 6.9, and 13.8 lb/ft^3, respectively, for the four treatments (Table 1). Because each tank contained one replicate of each treatment, all tanks contained the same number of fish. The water flow to each rearing tank was 15 gal/min of degassed well water (102% total gas saturation) at a temperature of 47.3°F. The flow rate was calculated (Liao 1971) to provide an estimated minimum dissolved oxygen of 7 ppm, which is above the incipient limiting level for salmonids (Davis 1975). Circular rearing units were chosen because of their lack of a water quality gradient (Burrows and Chenoweth 1970; Westers and Pratt 1977). Removal of water quality influences from test results were further assured by assigning cage positions, relative to the point of water inflow, at random in each rearing tank (Figure 1).

Dissolved oxygen concentrations were measured periodically around and within each cage with a polarographic meter or by the Winkler method with azide modification (APHA et al. 1971). Un-ionized ammonia levels were not measured, but were predicted (Soderberg, in press) to remain below 0.0003 ppm, which is much less than 0.0125 ppm—the concentration above which rainbow trout growth was affected (Smith and Piper 1975).

Fish were fed the GR6-30 diet (U.S. Fish and Wildlife Service, Diet Testing Development Center, Spearfish, South Dakota) three times daily at rates determined from Haskell's (1959) equation with a theoretical feed conversion of 1.5 and an estimated growth rate of 12.7 temperature units/in (Dwyer et al. 1981).

Fish were sampled every 30 d and average length and weight for each treatment were recorded. Fish were added to or removed from each cage at this time in order to maintain the desired density indices. The test was concluded 97 d after the fish were introduced in the cages.

Results and Discussion

Dissolved oxygen concentrations remained above 8 ppm. Therefore, all treatment differences were attributed to fish density and not water flow.

Growth and mortality data were analyzed by analysis of variance and Duncan's multiple-range test was used to identify differences within treatments at the 0.05 level of significance. There were no significant differences in final fish length, weight, and condition factors among the treatments (Table 1). Thus, growth was not affected by density in the range of densities tested.

Mortality was higher at a density index of 2.0 than at the three lower densities (Table 1). Moribund fish from treatments experiencing mortality were presumptively diagnosed for coldwater disease, a common disease at our laboratory. Mortality figures for the four replicates at the highest density were 5%, 6%, 25%, and 65%; two of the four replicates experienced significant epizootics of myxobacterial disease. Fish experiencing epizootics were in cages adjacent to those without significant mortality (Figure 1). Therefore, we attribute this effect solely to spatial factors within the cages.

We conclude from this study that lake trout may be reared successfully at a density index of at least 1.0. The application to practical fish culture is that most lake trout hatcheries generally provide more space than is necessary for their production requirements. Fish rearing units at proposed hatcheries can be made smaller than those typical of existing facilities.

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Intensive Culture of Walleye Fry with Live Food and Formulated Diets

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Abstract.—Techniques enabling the intensive culture of walleye fry (*Stizostedion vitreum*) to fingerling size were developed. Initial steps involved rearing fry on live food in vertical upwelling and modified rectangular units for 30 d with a mean survival of 14.6 and 7.8%, respectively. Dispersal of fry in rearing units and initial loading rate appeared to be two variables affecting fry survival. Walleyes were reared intensively under three different feeding regimens. Those fed formulated diets survived a maximum of 39 d, even though significant numbers ingested the food particles; these fish failed to inflate their swim bladders. Walleyes fed brine shrimp and then converted to formulated diets, and those fed brine shrimp and zooplankton followed by conversion to formulated diets demonstrated a 3.9 and 6.0% survival through 65 d.

Walleye (*Stizostedion vitreum*) fingerling culture in the U.S. has depended upon extensive culture systems and generally has been limited to the production of 35–50-mm (1.4–2.0-in) fingerlings (Davis 1953; Huet 1970). Production of larger fingerlings has been difficult because of inherent problems in these programs, such as cannibalism and loss of plankton bloom. Extensive–intensive rearing programs have been developed to produce 100-mm (4.0-in) or larger fingerlings (Cheshire and Steele 1972; Nagel 1974, 1976; Nickum 1978; Beyerle 1979). Under this system, pond fingerlings are brought to the hatchery, reared intensively on formulated diets, and stocked as 100–150-mm fingerlings in September. Conversion to formulated diets by pond fingerlings is variable and affected, in part, by their size when stocked into intensive systems (Cheshire and Steele 1972; Colesante 1982).

Although a production program for larger walleye fingerlings is possible with an extensive–intensive system, not all hatcheries have ponds available for the extensive phase and production tends to be unpredictable; therefore, an intensive culture program would be an asset. To date, intensive culture of walleye fingerlings on dry diets has failed; previous efforts have demonstrated that walleye fry will ingest inert particles, including formulated diets, but invariably the fish succumb (Nickum 1978; Howie et al. 1980). Limited success has been obtained by starting fry on diets of brine shrimp, followed by zooplankton and finally formulated diets (Howie et al. 1980).

The objectives of this study were: (1) to determine if walleyes could be reared intensively on live food in vertical upwelling and modified rectangular units; (2) to determine the most practical initial density for fry in these rearing units; and (3) to determine the survival of walleyes presented various formulated diets immediately after yolk absorption (group 1), following brine shrimp feeding (group 2), and following brine shrimp and zooplankton feeding (group 3).

Methods

Rearing Conditions

Modified rectangular units.—Pilot experiments had indicated that walleye fry are photopositive and tend to congregate in areas of rectangular rearing troughs where the desired illumination is present. This essentially concentrated fry in a small volume of water (441 fry/L) even though the density of fry in the entire trough was only 50 fry/L; heavy mortality from cannibalism occurred even though brine shrimp nauplii appeared abundant. To disperse fry evenly, rectangular fiberglass rearing units 6.1 × 0.91 × 0.49 m (20 × 3 × 1.6 ft) were completely covered with black plastic except for a center, 3.1-m (10-ft) linear section. Even distribution of fry within this uncovered volume of water—1.36 m^3 (48 ft^3)—was only obtained after a fluorescent light fixture (two 40-W tubes) was suspended 1.2 m (4 ft) above the trough.

Vertical upwelling units.—Vertical upwelling units have been successfully used to initially raise striped bass (Lewis and Heidinger 1981). Replicas of these upwelling units were used in this walleye culture program except the dimensions were 0.59 m high × 0.82 m long × 0.82 m wide (1.9 × 2.7 × 2.7 ft); usable rearing space was 0.40 m^3 (13.8 ft^3). Because fry in these units were distributed evenly by upwelling water currents, lights were not necessary to disperse fry; however, two fluorescent

FIGURE 1.—Modified rectangular rearing unit used in the intensive culture of walleye.

lights were used (four 40-W tubes) to facilitate cleaning operations.

Water flow and temperature.—Upwelling and rectangular units had a constant flow of 20.8 L/min (5.5 gal/min). When dry food was introduced, the flow in rectangular units was increased to 37.9 L/min (10 gal/min); it was not possible to increase flows in upwelling units. Water temperatures were not allowed to go below 14.5°C (58°F) or above 18.3°C (65°F) during the early rearing period. When ambient creek temperature was above 15.6°C (60°F) heated water was not used.

Fish loading.—Previously used initial loading rates of 14 fry/L (53 fry/gal) (Howie et al. 1980) appeared too low for a production program; therefore, during the 2 years of this study, modified rectangular rearing units were stocked with 20 to 54 fry/L (76 to 204 fry/gal) and upwelling units received 27 to 53 fry/L (102 to 200 fry/gal). The numbers of fry placed in each rearing unit were estimated by volumetric displacement.

Screens.—Vertical upwelling and rectangular units had screens with 19.7 meshes/cm (50 meshes/in) and were made of stainless steel or coated bronze. The openings in these screens were less than 200 μm which kept fry, and most brine shrimp and zooplankton, confined. When formulated diets were introduced, standard window screen with 4 meshes/cm (10 meshes/in) was used. This practice may have facilitated the conversion of walleyes to dry food because brine shrimp and zooplankton would pass through the larger mesh and only be available for a short time while formulated diets were available at 5-min intervals.

Feeding and Feeders

Walleye fry were presented food (either formulated diets or brine shrimp) 62 h after hatch.

Brine shrimp.—Initially, 28 g or approximately 6,272,000 brine shrimp cysts were incubated at 28°C (82°F) in May–Sloan jars containing a solution of 37.5 g/L salt (NaCl). Mean hatch from each jar was 56.7%.

Each upwelling and rectangular unit that received live food was equipped with one and two automatic feeders, respectively. These administered a slurry of brine shrimp every 9 min for 1.3 s during 0700–1600 hours and every 9 min for 0.45 s during 1600—0700 hours. Each feeder received 2.5 jars of shrimp nauplii from the incubator per day; thus fry in upwelling units received approximately 8,900,000 nauplii per day while those in rectangular units received 17,800,000. More nauplii were fed to fry in rectangular troughs because the nauplii settled to the bottom of these rearing units and became unavailable to the fry; in upwelling units, brine shrimp did not settle out. Fry in rectangular units were fed brine shrimp nauplii through a 3-m (10-ft) section of 10.1-cm (4-in) drain pipe, which was capped on both ends and had 3.2-mm (⅛-in) holes drilled every 15.24 cm (6 in) along the entire length. The drain pipe was suspended lengthwise, 15.24 cm (6 in) above the center of the trough. Brine shrimp were introduced at the upper end and at the midpoint of the pipe (Figure 1). A flow of 4 L/min (1.06 gal/min) of water pushed nauplii into the trough. Brine shrimp were supplied to upwelling units near the center of each unit.

Weaning from brine shrimp to formulated diets or zooplankton was accomplished in four stages over a 21-d period. On day 32 following hatch, the 2100 hour filling of the feeders was eliminated, but the 24-h feeding schedule was maintained; this reduced the total shrimp available to the fry by 12.5%. This was followed by a reduction in the amount of cysts incubated per jar to 14 g on day 35, resulting in a 60% total reduction of available shrimp. On day 42, only 7 g of cysts were incubated per jar, resulting in an 80% total reduction of shrimp, and finally brine shrimp were eliminated on day 53. Formulated diets were introduced on day 16 to group 2 fry and zooplankton was introduced on day 18 to group 3 fry.

Zooplankton.—Zooplankton collections were made in Oneida Lake with two, 1-m-diameter (3.3-ft) plankton nets with 243 μm mesh. Predominate species in these collections were *Diaptomus minutus, Cyclops bicuspidatus, Daphnia galeata, Bosmina longirostris,* and *Daphnia pulex.* The size range of the zooplankton was 0.5–2.5 mm in length, compared to 0.40–0.75 mm for brine shrimp. Collections were filtered to remove incidental fish and were placed in a holding trough. Equal volumes of zooplankton were added to walleye rearing units twice daily. We did not try to quantify the amount of zooplankton fed; however, more than adequate amounts appeared to be present during visual inspections. Weaning from zooplankton to formulated diets occurred over 13 d. During this period, a single day's collection was made to last 2 d, then 3 d and so on until no further zooplankton was added. Formulated diets were introduced on day 38 to group 3 fish.

Formulated diets.—Three dry diets were tested: Abernathy Salmon Diet, produced by Zeigler Brothers of Gardner, Pennsylvania; W-16 diet developed and produced by Spearfish Diet Development Center (U.S. Fish and Wildlife Service); and Zeigler's (shrimp-colored) Larval Diet (AP-100) developed and produced by Zeigler Brothers. Formulation specifications for the Abernathy and W-16 diets are given in Table 1. Proximate analysis for the closed formula AP-100 diet was 45.8% protein, 10.3% fat, 2.6% fiber, 9.5% ash, and 8.0% moisture.

Fry in group 1 that received only W-16 or Abernathy diet were fed starter (420–595 microns) from circular type feeders (manufactured by Mo-Do Enterprises, Rancho Cordova, California); they were fed every 5 min for a 15-s duration, 24 h/d.

Zeigler's AP-100 Larval Diet was available in five sizes: #0000 (150 μm), #000 (200 μm), #00 (250 μm), #0 (350 μm), and starter (500 μm). Because this feed was so fine, conventional feeders would not work. A vibrator feeder with a blower (Sweeney Enterprises) worked well. Walleye fry fed only AP-100 received equal parts of #00, #000, and #0000 every 9 min, 24 h/d. Approximately 1.1 g of AP-100 were administered at each cycle. All formulated diets were fed in excess as evidenced by accumulations of unused feed on the bottom of rearing units.

Walleyes fed brine shrimp followed by formulated diets (group 2), or brine shrimp and zooplankton followed by formulated diets (group 3), in upwelling units were provided a mixture of standard starter and number one crumbles (between 595 and 841 μm) every 5 min for 15 s, 24 h/d from circular feeders. Walleye in rectangular troughs were fed a mixture of starter and number one crumbles from Loudon trough feeders (manufactured by North Star Company, Gaston, Oregon) at the same feeding frequency as those in upwelling units.

TABLE 1.—Formulation specifications (% total weight) for Abernathy and W-16 diets.

Ingredient	Diet	
	W-16	Abernathy
Herring meal	50	58[a]
Whey product (dried)		10
Blood flour (dried)	20	5
Soy flour	10	
Shrimp meal (dried)	5	5
Wheat middlings		remainder[a]
Brewers yeast		5
Vitamin premix #2		1.5
Vitamin premix #30	0.6	
Choline chloride 60%		0.58
Choline chloride 50%	0.225	
Ascorbic acid	0.13	0.10
Trace minerals	0.025	0.0005
Pellet binder	2	2
Fish oil	12	12[a]

[a] Fish meal may be decreased depending upon protein content, and the quantity of added fish oil may be adjusted to meet final product guarantee. Wheat middlings are added to compensate for these changes.

Results

Walleye survival, through day 30, on live food diets averaged 14.6% in upwelling units and 6.7% in troughs (Table 2). Although replicated experiments were not conducted, the data suggest that survival was better at lower initial loading rates in both types of rearing units. Fingerlings remaining after 30 d were 27.3 mm (1.07 in) in length.

Walleye fry in the three upwelling units that received only formulated diets 62 h after hatch (group 1) were first observed with feed in their gut on day 7. Analysis of a random sample of 20 fry from each of three units indicated that 10% were ingesting W-16 and 5% were ingesting both Abernathy and AP-100. The ingestion rate varied around these levels until day 12, when 50% of the surviving fry had feed in their gut. On day 14, 55% of the remaining fry were ingesting W-16, 60% Abernathy, and 75% AP-100. Although there was a high ingestion rate in those fish fed AP-100, their numbers declined very rapidly and this treatment

Table 2.—Survival of walleye fry reared intensively under different feeding regimens and loading densities.

Feeding regimen	Density fry/unit (fry/L)	Rearing period (d)	Survival (%)
Upwelling unit			
Brine shrimp–zooplankton	10,750 (27)	30	20.4
Brine shrimp–zooplankton	21,000 (53)	30	8.8
Mean			14.6
Brine shrimp–Abernathy	20,000 (50)	65	3.9
Brine shrimp–W-16	20,000 (50)	65	5.6
Brine shrimip–AP-100	20,000 (50)	65	2.3
Mean			3.9
Brine shrimp–zooplankton–Abernathy	20,000 (50)	65	7.9
Brine shrimp–zooplankton–W-16	20,000 (50)	65	4.8
Brine shrimp–zooplankton–AP-100	20,000 (50)	65	5.2
Mean			6.0
Rectangular unit			
Brine shrimp only	30,000 (20)	30	11.6
Brine shrimp–zooplankton	30,000 (20)	30	9.7
Brine shrimp–zooplankton	53,750 (35)	30	3.7[a]
Brine shrimp–zooplankton	20,000 (13)	30[b]	11.7
Brine shrimp–zooplankton	40,000 (20)	30[b]	1.9[a]
Mean			6.7

[a] *Columnaris* disease diagnosed and responsible for high losses in these fish.

[b] Thirty-day survivors in these groups were combined and converted to Abernathy diet in a modified rectangular trough. 32.9% of the fingerlings converted.

was terminated on day 18 when only 550 fish remained in very poor condition. On day 18, an estimated 2,000 fry fed Abernathy diet were transferred to the upwelling unit receiving W-16 because that diet appeared to be producing the best results in terms of survival. After combining these lots, periodic samples indicated all surviving fish were ingesting W-16; however, losses continued and on day 39 this phase of the study was terminated with 130 surviving fish. Length gains during this period had been minimal.

Fry in the three upwelling units that received brine shrimp and formulated diets (group 2) and fry in the three units that received brine shrimp, zooplankton, and formulated diets (group 3) were first observed ingesting brine shrimp on day 6 following hatch. On day 12, 100% of the fry in these units had brine shrimp in their gut. On day 29, 13 d following the addition of dry diets to group 2, stomach analysis indicated 92.3% of the surviving fish were feeding on brine shrimp and 12.3% were eating dry diets alone or dry diets and brine shrimp. Also on day 29, 11 d following the addition of zooplankton to group 3, stomach analysis showed 97.8% of the fish were still feeding on brine shrimp and only 2.2% were feeding on zooplankton. This indicated that the conversion to dry diets or zooplankton from brine shrimp was equally difficult for walleyes reared under these conditions.

Sampling and counting of surviving fish was conducted on day 65. Fish in upwelling units of groups 2 and 3 were 36.5 mm long (range 35.8–37.0 mm). Fingerlings in group 2 had completed the conversion to formulated diets with a mean survival of 3.95%; fish weakened from starvation were no longer present. Those in group 3 had undergone major losses by day 65, but some weakened fish remained; these were easily identified, discarded, and not included in the survival data; mean survival in this group was 5.97% (Table 2).

Cannibalism did not appear to be responsible for the major losses experienced in this study. Between days 10 and 12, active cannibalism occurred in all rearing units. Removal of cannibals during this period reduced the incidence of attack. It appeared that some fish were inherently cannibalistic and once they were removed, new cannibals rarely developed.

Discussion

Early efforts indicated that intensive culture of walleye fry on live food was possible in both modified rectangular and upwelling units. The major advantages of upwelling units were ease of cleaning, desired fry distribution without special lighting, more efficient use of brine shrimp by the fry, and simpler routine maintenance. In modified rectangular units, it appears that high initial loading densities promoted disease, particularly *Columnaris*. Low water exchange rates (0.6/h) could have contributed to disease outbreaks.

Fry survived longest on the W-16 diet; therefore, it seems most promising for future studies. However, another factor that had a major impact on survival of fish in group 1 was their failure to inflate swim bladders. Difficulties with the survival of cultured striped bass (*Morone saxatilis*), sea bass (*Dicentrarchus labrax*), and red sea bream (*Pagrus major*) have been attributed to problems with swim bladders (Doroshev 1970; Kitajima et al. 1981; Johnson and Katavic 1984). Few fry were found with inflated swim bladders in units presented only formulated diets; this became obvious on day 12 after inspection of stomach contents. All surviving fish in the six upwelling units receiving brine shrimp had inflated their swim bladders. There was an obvious heavy surface film present in upwelling units receiving formulated feed. If walleyes must take in air to inflate their swim bladder, this may have prevented the process. This heavy film was not present in other upwelling units that received brine shrimp. An air stone was put below the water's surface in group 1 upwelling units. This dispersed the film, but no fish were observed with inflated swim bladders. It is not known if losses in group 1 were caused by inadequate nutrition, failure to inflate swim bladders, or a combination of both.

The brine shrimp–formulated diet feeding regimen produced intermediate survival; the W-16 diet produced the best results in this group. Although Zeigler's AP-100 larval diet was very similar in color to brine shrimp nauplii, dispersed well in water, and seemed nutritionally balanced, survival was poorest on this diet.

The brine shrimp–zooplankton–dry diet feeding regimen (group 3 upwelling units) produced the best fingerling survival through day 65, almost 50% better than group 2; Abernathy diet produced the best results (7.9% survival). It appears that, although zooplankton is not necessary for conversion of walleyes to formulated diets, it is certainly beneficial at this point.

Walleye can be reared from the fry stage to large fingerling size under intensive conditions. Factors limiting the large-scale production of fingerlings are brine shrimp and zooplankton availability and the apparent need for low fish densities in the rearing units. Commercial sources of brine shrimp have been expensive and sometimes unreliable, and it appears that shrimp nauplii are wasted in present feeding programs—it is possible that brine shrimp can be fed for a much shorter period of time. Zooplankton in the diet of walleye fingerlings appears to enhance their conversion to formulated diets, and large-scale harvest of zooplankton has been done successfully (Shafland et al. 1979) from natural waters. These techniques could be integrated into a fingerling program. Initial fry densities in the various experimental rearing units were so low that, coupled with low survivals, a production level program is presently precluded. Further testing and modifications of rearing units and feeding techniques should allow higher fry densities and should increase survival throughout the various rearing stages.

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Behavioral and Biochemical Correlates of Hatchery Rearing Methods on Lake Trout

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Abstract.—Five-month-old lake trout (*Salvelinus namaycush*) were reared in covered or uncovered rearing troughs and fed by hand or from a "demand" feeder from June 25 to September 14 under hatchery rearing conditions. The covered, hand-fed fish were significantly heavier and more active when startled. Hand-fed fish were generally lighter in color, heavier in weight, and dominated the bottom of a test cylinder. Fish in the covered rearing troughs had increased startle reactions to a visual stimulus, increased body and whole brain weights, and reduced brain serotonin levels. The poorest food conversion ratios were found in the uncovered, hand-fed group. The results reveal both neural and behavioral sensitivity to hatchery rearing conditions in lake trout, and suggest improvements of fish hatchery rearing methods.

Fish behavior can be altered by hatchery rearing methods, thereby influencing survival rates once the fish are stocked. Ritter and MacCrimmon (1973) found that rainbow trout (*Salmo gairdneri*) raised in ponds from 5 to 11 months of age were more wary than those continuously reared in laboratory tanks. Covering fish-rearing troughs may increase the startle response in hatchery-reared fish such as lake trout (*Salvelinus namaycush*). It is possible that fish reared in uncovered troughs adapt to the presence of humans and this behavior might transfer to predators in general making the stocked fish less likely to startle and flee. A number of experiments have demonstrated that lighting conditions can affect activity in fish (Jones 1956; Hoar et al. 1957; Kwain and MacCrimmon 1967, 1969). Raising hatchery fish in covered troughs also enhances body growth (personal observation) and may affect brain development because lighting is correlated with diurnal central nervous system (CNS) catecholamine (Sauerbier and Meyer, 1977; LeBras 1979) and serotonin concentrations (Genot et al. 1981).

Hatchery feeding methods may also influence fish behavior. Traditional methods involve hand feeding, but advances in automation have led to the use of automatic and demand-type feeders. Post-stocking survival rates may be enhanced by demand-type feeding because the fish must act to initiate food delivery by striking a small ball to release food, and exposure to humans is almost eliminated. Furthermore, this method may increase aggression in fish because the food is distributed to one small area (Schreck 1981).

A number of behaviors are correlated with CNS biogenic amines in teleosts. Activity levels of fish have been correlated with diurnal fluctuations of CNS dopamine, norepinephrine (LeBras 1979), and serotonin concentrations (Genot et al. 1981). Catecholamines have also been hypothesized to play a role in feeding behavior (Bray 1974; Peter 1979); emotional behaviors, such as avoidance and startle responses (Plaznik and Kostowski 1983); and aggressive, territorial behavior (McIntyre et al. 1979).

Lake trout have received little attention related to the identification of monoaminergic nerves in the peripheral and central nervous systems (Santer 1977). Even less attention has been paid to the behavioral correlates of biogenic amine concentrations in lake trout. Some published research is available on rainbow trout. For instance, McIntyre et al. (1979) reported decreased CNS norepinephrine and increased dopamine in dominant rainbow trout when compared to more frequently attacked fish. This observation led to their suggestion that dopamine and norepinephrine may play a role in the dominance hierarchy of rainbow trout. Fish coloration is also associated with territorial dominance (Howard 1974; McIntyre et al. 1979). Lighter colored fish are reported to be more dominant. Lake trout do not appear to show this relationship, possibly because they are not as aggressive as other salmonids (Noakes 1980). It is possible that aggressive behavior might be enhanced in lake trout by specific rearing methods.

In this experiment we examined the behavioral and neurochemical effects of different hatchery rearing methods on lake trout fingerlings. Two, two-level variables were manipulated: (1) the fish were reared in either covered or uncovered rearing troughs, and (2) the fish were fed either by hand or by demand feeders. Effects of the four different regimes on biogenic amines, whole brain weights, body weights, activity, startle response, color, and dominance behavior were evaluated.

Methods

Two replicates of 400, 5-month-old Lake Manitou lake trout were randomly selected from the production lot at the Provincial Fish Hatchery, North Bay, Ontario. The average weight of the fish was 1.87 g, sex was undetermined. The fish were randomly allocated to one of four treatment groups in equal numbers: group 1 was reared in an uncovered trough and was hand-fed; group 2 was uncovered and fed from the CYAQ-7 Pendulum (demand) Feeder developed by the Ontario Ministry of Natural Resources; group 3 was covered and hand-fed; and group 4 was covered and demand-fed. The fish were reared in their respective treatment groups from June 25 to September 14 under otherwise normal indoor hatchery rearing conditions.

Each group was held in a separate rearing trough 56 cm long × 55.5 cm wide × 19 cm deep. Plywood sheeting placed over the troughs provided complete protection from above but allowed some light to diffuse in from the ends. All groups received the same water flow—4.54 L/min, 4.61 changes/hour, at 9.25 ± 3.25°C. The spring water used at the hatchery had an average pH of 6.5, and 9.5 mg/L oxygen.

The fish were fed 1.5 granule fish food for the first 6 weeks and then 2 granule food either by hand or by demand feeder. Hand-feeding consisted of eight feedings per day, once per hour from 1000 to 1700 hours, between June 25 and August 17, and seven times per day for the rest of the period, and provided 1–2% body weight of feed per day. Six days before behavioral testing the fish were fin-clipped for identification purposes.

The behavioral testing was conducted on five consecutive evenings between 1800 and 2300 hours. Twenty lake trout were randomly selected from each of the two covered or uncovered tanks. Activity and startle-response testing were carried out in a circular apparatus with an exterior wall circumference of 85 cm and interior wall circumference of 58 cm, which provided a 4.3-cm-wide circular runway. The runway was marked with eight equidistant lines to allow measurement of distance travelled. Gently flowing water (90 mL/min) filled the runway to a depth of 4 cm and provided six water changes per hour. The water temperature was 8.15 ± 0.65°C. Each fish was placed in the runway at the same start position; the order of group testing was randomized. Activity was recorded as the number of lines crossed by a fish every 15 s for 45 s. The fish were then startled with a visual stimulus (a cut-out bird silhouette) and then an "auditory" stimulus (a vibration induced by a 50-g pendulum striking the side of the runway). Following each stimulus, the activity of the fish was again recorded for three consecutive 15-s intervals.

Dominance testing was carried out by placing pairs of fish in a 9-cm diameter glass cylinder marked off in 1.5-mm gradations. Both the cylinder and the aquarium in which the cylinder was placed were filled with water to a height of 37.5 cm. Pairs of fish were chosen by random selection based on a four by four matrix defined by the treatment groups. All possible combinations were tested. Immediately prior to the dominance testing, the fish were compared to identify the lighter and darker fish of each pair. They were then placed in the cylinder and observers recorded the distance of each fish from the bottom of the cylinder at 30-s intervals for 3 min. Each fish was then weighed and released.

Ten untested fish were randomly selected from each of the treatment groups for neurochemical assay. They were weighed and decapitated, and their whole brains were placed in capsules and frozen in dry ice. The brains were assayed by fluorescence methods (Pappas et al. 1978). All behavioral testing and neurochemical assays were conducted by personnel who were unaware of the treatment group being tested.

Results

Three separate multivariate analyses of variance (MANOVA; Harris 1975) were carried out: the assay-related results, the activity–startle test results, and the dominance-test results. The assay results and the brain weights are summarized in Table 1. The fish from uncovered troughs had lower brain weights and tended to have higher serotonin levels than the fish from covered troughs (Wilk's lambda = 0.834; $s = 1$, $m = 1$, $N = 24\frac{1}{2}$, $P = 0.05$). No other effects were significant.

TABLE 1.—Mean whole-brain concentrations (±SD) of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) and mean brain weights of lake trout that were hand- or demand-fed in uncovered or covered troughs ($N = 10$ fish per treatment).

Treatment	NE (ng/g)	DA (ng/g)	5-HT (ng/g)[a]	Brain weight (mg)[b]
Uncovered troughs				
Hand-fed	418±59	1,652±424	364±60	52±8
Demand-fed	412±64	1,602±278	369±31	53±12
Covered troughs				
Hand-fed	387±88	1,687±737	348±45	59±9
Demand-fed	420±151	1,426±546	340±121	54±8

[a] $F(1,54) = 3.77$, $P = 0.057$.

[b] $F(1,54) = 8.76$, $P < 0.05$

TABLE 2.—Activity scores for lake trout raised in uncovered and covered troughs before (baseline) and after visual and auditory startle stimuli. Activity was measured during three consecutive 15-s periods. Values are means ± SD for 40 fish; the numbers of significant multiple comparisons are in parentheses (Tukey's test; $P < 0.05$).

Treatment	First 15-s interval[a]	Second 15-s interval[b]	Third 15-s interval
Uncovered troughs			
Baseline	10.1±7.40 (1)	3.7±4.67 (1)	3.2±5.11
Visual	14.4±12.03 (1)	2.7±4.90 (1)	0.4±1.65
Auditory	6.7±7.18 (3)	2.3±4.11 (1)	2.3±4.37
Covered troughs			
Baseline	12.5±8.58 (1)	2.2±3.34 (1)	2.5±3.76
Visual	28.0±9.37 (5)	6.5±7.02 (5)	1.6±3.40
Auditory	4.8±5.75 (4)	2.3±3.55 (1)	1.7±3.59

[a] $F(2,228) = 18.35$; $P < 0.05$.

[b] $F(2,228) = 6.73$; $P < 0.05$.

The lake trout from covered troughs became significantly more active following the visual stimulus than the fish from uncovered troughs (Wilk's lambda = 0.842; $s = 2$, $m = 0$, $N = 112$, $P < 0.05$). This increased response to the visual stimulus was still evident during the second 15-s interval (Table 2).

The hand-fed fish raised under cover were significantly more active than the demand-fed fish raised under cover (Wilk's lambda = 0.842; $S = 1$, $m = \frac{1}{2}$, $N = 112$, $P < 0.05$). This observation was significant during the second activity interval (Table 3).

The effects of startle method, feeding method, and cover manipulation were all significant. The visual startle-stimulus increased activity, while the auditory stimulus tended to reduce activity; both demand-feeding and covering were associated with increased activity.

The analysis of dominance test data, fish color and body weight revealed a significant effect only for feeding (Wilk's lambda = 0.826; $s = 1$, $m = 3$, $N = 93\frac{1}{2}$, $P < 0.05$) and cover (Wilks's lambda = 0.844; $s = 1$, $m = 3$, $N = 93\frac{1}{2}$, $P < 0.05$). The hand-fed fish were significantly heavier (7.59 g) than the demand-fed fish (6.64 g) and were also judged to be significantly lighter in color. Furthermore, between the second and third measurement in the cylinder, the hand-fed fish moved significantly closer to the bottom of the cylinder than the demand-fed fish, changing on the average from 3.81 cm to 2.56 cm, while the demand-fed fish changed from 3.14 cm to 4.47 cm. No other effects were significant.

Covering the rearing trough significantly affected the body weights of the lake trout; the covered fish averaged 7.80 g and the uncovered ones 6.43 g. Also, the mean position in the cylinder was affected significantly by the cover variable; uncovered fish held a lower position in the cylinder than the covered at all measurement periods. No other tests were significant.

At the end of the experiment all fish were weighed and food conversion ratios were calculated for each group. Analysis of variance of the final weights after approximately 3 months of treatment revealed a significant feeding × cover interaction ($F[1,783] = 6.71$, $P < 0.05$). The mean weights of the four treatment groups were as follows: uncovered hand-fed fish, 6.47 g ($N = 191$); uncovered demand-fed fish, 6.16 g ($N = 199$); covered hand-fed fish, 8.62 g ($N = 199$); and covered demand-fed fish, 7.56 g ($N = 198$). The uncovered, demand-fed fish were significantly lower in body weight than the fish raised in covered troughs and the covered, hand-fed fish were significantly heavier than the fish raised in uncovered troughs. No other comparisons were significant (Tukey's test $P < 0.05$).

Food conversion ratios were calculated for the four treatment groups as a ratio of total amount of food fed to the total weight gained by the treatment group. The uncovered, hand-fed group had the least efficient food conversion of 1.56, while the covered, hand- and demand-fed groups had food conversions of 1.02 and 1.09, respectively; the uncovered, demand-fed fish had a food conversion of 0.99.

Discussion

Long-lasting behavioral and neural sensitivity to environmental rearing conditions has been consistently demonstrated to occur in mammals (Krech et al. 1966; Rosenzweig et al. 1970; West and Greenough 1972; Greenough 1975; O'Shea et al. 1983). This phenomenon has also been demonstrated in teleosts, specifically rainbow trout, but lake trout may not reveal the effect (Ferguson et al. 1983). In this experiment we have demonstrated that alternative rearing practices of a fish hatchery were accompanied by behavioral and neural changes in lake trout. The results also suggest improvements that may be made in hatchery rearing practices.

The two main manipulated variables, cover and feeding method, interacted on three of the dependent measures: total body weights, food conversion, and startle response to a visual stimulus. The covered, hand-fed fish were significantly heavier and were more active when startled with a bird silhouette than the other groups. They also had ideal food conversion, while the uncovered, hand-fed fish appeared to be less efficient.

Covering the rearing troughs appeared to alter the startle response of lake trout fingerlings; the covered fish became more responsive to the stimulus and heavier in body weight regardless of the feeding method. Covering also resulted in an increase in whole brain weights and a decrease in serotonin levels.

The uncovered fish tended to seek lower levels in the cylinder test, as did the hand-fed fish. The hand-fed fish, which tended to dominate the bottom of the cylinder, were both heavier in weight and lighter in color than the demand-fed fish. One should note, however, that coloration does not appear to play a role in dominance behavior in lake trout (Noakes 1980). More research is required to evaluate the behavioral implications of the cylinder test. The "forced" nature of this test may lead to clearer measurement of dominance behavior in lake trout.

The above results demonstrate that lake trout fingerlings respond behaviorally, neurally, and anatomically to different rearing methods. The behavioral and theoretical implications of the CNS alterations require further study because very little is known about the behavioral correlates of biogenic amines in lake trout. The alterations provide support for the suggestion that the behavioral changes observed in this experiment have a neural basis and may be long lasting. This conclusion warrants an examination of the Ferguson et al. (1983) finding that behavioral plasticity in lake trout is quite restricted. The major difference in the methodology of the two experiments may be the time allowed to adapt to the altered environment. It is quite likely that the 48-h adjustment period provided in the Ferguson et al. experiment is not sufficient to lead to long-term behavioral changes and may only reflect short-term adjustments to altered environmental conditions.

TABLE 3.—Activity scores for lake trout reared in covered and uncovered conditions and fed either by hand or from demand feeders. Activity was measured during three consecutive 15-s periods. Values are means ± SD; numbers of significant multiple comparisons are in parentheses (Tukey's test; $P < 0.05$).

Treatment	*N*	First 15-s interval	Second 15-s interval[a]	Third 15-s interval
Uncovered troughs				
Hand-fed	57	9.1±9.20	2.4±3.86 (1)	1.9±4.14
Demand-fed	63	11.5±9.87	3.4±5.11 (0)	2.0±4.14
Covered troughs				
Hand-fed	60	12.6±10.84	4.5±5.82 (1)	2.4±3.84
Demand-fed	60	17.6±13.69	2.8±4.61 (0)	1.5±3.27

[a] $F(1,228) = 4.91$; $P < 0.05$.

Evaluation of our results from the hatchery view point provides some important suggestions for hatchery rearing methods. Although data after stocking are not available for the different treatment groups, we predict that a fish is more likely to survive after stocking if it responds to a bird-shaped shadow and is heavier in body weight at stocking time. Considering these factors, the poorest rearing method appears to be hand-feeding fish in uncovered troughs.

The effect of these rearing methods on post-stocking survival rates in lake trout will be of great importance. We suspect that the behavioral and neural alterations will be long-lasting and may parallel the plasticity observed in mammals following environmental manipulation.

Acknowledgments

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Effect of Exercise on Feed Consumption, Growth, Food Conversion, and Stamina of Brook Trout

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Abstract.—This study compared the practical effects of rearing brook trout (*Salvelinus fontinalis*) in units either with or without water currents sufficient to force them to swim actively and continuously. After 10 weeks, exercised fish fed to satiation were heavier than both the unexercised fish fed to satiation and the exercised fish fed the same percentage body weight per day as unexercised fish. The unexercised fish and the exercised fish fed equal percentages of body weight showed no difference in final weight. Fish in both exercised groups had greater swimming stamina than unexercised fish at the end of the study. Food conversions for the exercised fish fed to satiation, the unexercised fish fed to satiation, and the exercised fish fed at equal percentages of body weight as unexercised fish were 1.54, 1.58, and 1.49, respectively.

Characteristics that constitute high-quality, hatchery-produced juvenile salmonids, destined for release into the wild, are not unanimously agreed upon by managers and researchers. Vincent (1960), Poston et al. (1969), Burrows (1969), Wendt and Saunders (1972), and Cresswell and Williams (1983) reported improved survival or ability to combat stress in groups of salmonids that had been intentionally exercised for varying periods of time prior to release. In contrast, Horak (1972) disputed the value of stamina testing for trout that were to be placed in lakes and streams for sportfishing.

This study was conducted to measure the effects of forced exercise and level of feeding on the growth, food conversion, and swimming stamina of juvenile brook trout (*Salvelinus fontinalis*).

Methods

On 24 May 1972, 400 brook trout averaging 3.7 g (0.13 oz) each were placed into each of 12 fiberglass, circular rearing units having an inside diameter of 84 cm (33 in) and a mean water depth of 30 cm (11.8 in). The water volume (minus the standpipe volume) of each unit was approximately 160 L (42 gal). The 8–9°C (46.4–48.2°F) fresh water entered each tank at a rate of 11.3 L/min (3 gal/min) via three holes drilled perpendicularly into a 2.5-cm (1-in) horizontally oriented polyvinyl chloride pipe. Incoming water was introduced at the surface and exited from the bottom of the unit through two concentric standpipes—the outer one passing water through perforations around its lower 10 cm (4 in). The standpipe design helped to continuously remove fecal and other waste products from the units. The incoming water was filtered to ensure that the fish received no natural food.

After a 2-week acclimation period under uniform conditions with no directed water currents in the units, the experimental treatments were begun. The design incorporated three treatments, each replicated four times. In the eight units with fish to be exercised, incoming water flow was directed at an angle to the water surface so that a current velocity of 11 cm/s (4.3 in/s) was generated, as measured ⅓ of the radius from the perimeter of the tanks. After 5 weeks, the current was increased to approximately 15 cm/s (6 in/s) in the eight tanks of exercised fish. In the four units of unexercised fish, the incoming water was baffled by an aluminum container suspended beneath the three holes delivering water so no measurable current was produced (i.e., the fish did not have to actively swim to hold their position in the rearing unit).

All fish were fed Rangen's trout diet (Zeigler Brothers, Incorporated, Gardners, Pennsylvania); four units each of exercised and unexercised fish were fed to satiation five times per day. When several food pellets remained on the bottom of the tank for more than a minute, the fish were considered satiated. The other four units of exercised fish were fed, as nearly as possible, the same percentage of their body weight per day as the unexercised fish (i.e., they were not fed to satiation).

Determining the amount to feed the exercised–unsatiated fish required calculating the percentage of body weight to feed per day. During the first 2

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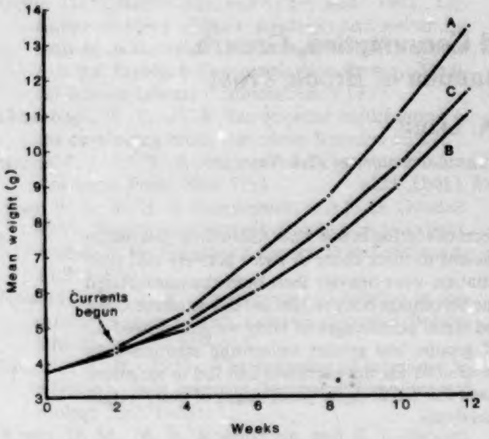

FIGURE 1.—Comparison of growth of brook trout that were (A) exercised and fed to satiation, (B) not exercised and fed to satiation, and (C) exercised and fed the same percentage of body weight per day as fish in treatment B.

d of the treatments, exercised–unsatiated fish were fed the same percentage of their body weight as had been eaten by unexercised–satiated fish during the 2 d prior to the initiation of the trial. Throughout the study, the 2-d rations for exercised–unsatiated fish were determined after calculating the previous 2-d ration in percentage of body weight for unexercised–satiated fish. Because fish were weighed every 2 weeks, the mean fish weights on any particular day between the days of weighing were not known. Without corrections for weight gains during these 14-d intervals, there would have been significant errors in calculating the percent of body weight per day eaten by unexercised–satiated fish. Therefore, during the first 4 weeks after the treatments began, a 10% increase in mean body weight was assumed at the midpoint of each 2-week interval. From previous experience at this laboratory, this assumption approximates actual increase in weight. To further reduce this source of error, the growth of unexercised–satiated and of exercised–unsatiated fish was calculated by linear regression of the form $\log_{10}y = a + bx$ after three data points were obtained. A new regression formula was calculated at the end of each 2-week period. From the regressions, an estimate could be made of the mean weight of unexercised–satiated and exercised–unsatiated fish for any day during the interval; then the percentage of body weight eaten by unexercised–satiated fish and, therefore, the amount to be fed to exercised–unsatiated fish could be determined.

After 10 weeks of treatments, the swimming stamina of fish in two replicates from each treatment group was tested by modifications (Leon, unpublished) of the method developed by Thomas et al. (1964). The modifications were as follows. (1) Only fish that continued to swim in the testing device one-half minute after the fish-retaining screen was removed were measured for swimming ability; those leaving during the first half-minute were considered invalid for stamina testing. (This modification drastically reduced the variance between replicates.) (2) Swimming performance was expressed as distance traveled in order to eliminate analysis problems created by the increasing water velocity increments in the device.

TABLE 1.—Means of four replicates for each of three treatments in respect to food fed, weight gain, and food conversion for 14-d intervals with brook trout at 8–9°C (46.4–48.2°F).

	Exercised and satiated					Unexercised and satiated					Exercised and unsatiated				
Week	Feed/ fish (g)	% body weight fed/d	Fish weight (g)	2-week gain (g)	Feed/ gain	Feed/ fish (g)	% body weight fed/d	Fish weight (g)	2-week gain (g)	Feed/ gain	Feed/ fish (g)	% body weight fed/d	Fish weight (g)	2-week gain (g)	Feed/ gain
0			4.42					4.41					4.35		
2	1.73	2.50	5.48	1.06	1.63	1.06	1.61	4.99	0.58	1.83	0.96	1.44	5.15	0.80	1.20
4	2.31	2.63	7.06	1.58	1.46	1.75	2.26	6.05	1.06	1.65	1.76	2.17	6.44	1.29	1.36
6	2.74	2.48	8.71	1.65	1.66	2.01	2.15	7.33	1.28	1.57	2.17	2.16	7.88	1.44	1.51
8	3.36	2.43	10.97	2.26	1.49	2.59	2.27	9.00	1.67	1.55	2.75	2.25	9.57	1.69	1.62
10	3.89	2.27	13.51[a]	2.54	1.53	3.08	2.19	11.05[a]	2.05	1.50	3.29	2.21	11.69[a]	2.12	1.55
Total	14.03			9.09		10.49			6.64		10.93			7.34	
Mean[b]					1.54					1.58					1.49

[a] Pooled standard error for final mean weights = 0.22.

[b] Mean food conversion for 10 weeks was obtained by dividing total food fed by total gain, not by averaging each 2-week conversion.

Analysis of variance was used to test for significant treatment effects on growth (four replicates) and stamina (two replicates). Mean food conversions (amount fed divided by weight gain) were also calculated.

Results

Throughout the study it was obvious that exercised–satiated and exercised–unsatiated fish actively swam to hold their positions in the tanks. In contrast, unexercised–satiated fish milled about haphazardly, apparently exerting little muscular energy. Exercised–satiated fish grew at a faster rate for the 10 weeks, whereas unexercised–satiated and exercised–unsatiated fish showed parallel growth rates during the last 4 weeks (Figure 1). Exercised–satiated fish had greater ($P < 0.01$) final mean weights than unexercised–satiated and exercised–unsatiated fish (Table 1). There was no difference ($P > 0.05$) between unexercised–satiated and exercised–unsatiated fish weights. Exercised–satiated fish displayed greater ($P < 0.01$) swimming stamina than unexercised–satiated fish but not greater than exercised–unsatiated fish (Table 2). Exercised–unsatiated fish had greater ($P < 0.05$) stamina than unexercised–satiated fish. Mean food conversion values for the 10-week period were best for exercised–unsatiated fish and worst for unexercised–satiated fish. No statistical analysis was performed on this parameter because only the mean conversions for each treatment were available—the individual replicate conversion data were inadvertently lost.

Discussion

The data show that, under the experimental conditions, brook trout grew best when reared in a current velocity of 1.5–2.0 body lengths per second if fed to satiation. This agrees with the findings of Kuipers (1982) who obtained similar results with Atlantic salmon (*Salmo salar*). Furthermore, the data show that exercised fish will eat more than unexercised fish if given the opportunity. Although one might expect that the increased activity and greater food intake would have resulted in poorer food conversion, such was not the case. It is possible that the exercised–satiated fish were physiologically healthier, thus requiring less energy per unit of activity and for maintenance than the unexercised–satiated fish. The greater swimming stamina of the exercised fish supports this proposition. Poston et al. (1969) found that levels of muscle glycogen and lactate in exercised brook trout recovered faster than unexercised fish during swimming stress tests. Burrows (1969) noticed differences in ratios of mature and immature red blood cells, and Davison and Goldspink (1977) found enlarged mitochondria, in exercised fish—changes that could account for some of the increased swimming stamina and food conversion of exercised fish observed in this study. Another possible contributor to the better performance of the exercised fish was increased irrigation of the gills caused by the water current. This possibly reduced the energy required by the fish to absorb oxygen and excrete metabolites. However, considering the light loading of each unit (exchange rate of 4.3/h) and near oxygen saturation, I believe that the irrigation effect was less than the exercise effect.

TABLE 2.—Meters swum by brook trout in a stamina tunnel during two replicates for each of three treatments. Mean values followed by the same letter are not significantly different ($P > 0.05$).

Treatment	Distance swum (m)		
	Test 1	Test 2	Mean[a]
Exercised–satiated	1,029	985	1,007 z
Unexercised–satiated	842	876	859 y
Exercised–unsatiated	929	951	940 z

[a] Pooled standard error = 17.3.

Although it could have been anticipated that exercised–satiated fish would have better swimming stamina than unexercised–satiated fish, it was not expected that the exercised–unsatiated fish would be better swimmers than unexercised–satiated fish because they received less than their optimal caloric needs. Not only did they have more stamina than unexercised–satiated fish, but they also had the best food conversion of all treatments. Similar findings in respect to food conversion for fish fed to less than satiation have been commonly observed by others (Hugh Poston, Tunison Laboratory of Fish Nutrition, personal communication).

Surprisingly, there was no difference in mean weight between unexercised–satiated and exercised–unsatiated fish. That the latter were metabolically more efficient is corroborated by the lower food conversion.

Other researchers (Flagg et al. 1983) are currently studying the use of exercise to ameliorate stress in coho salmon (*Oncorhynchus kisutch*) when they are transferred to sea water. In subsequent work (unpublished), they found that smolts may take 2–3 weeks to regain their freshwater swimming stamina when put in sea water and that previously exercised fish did so in a shorter time. This difference could be manifested by differential susceptibility to predation.

Based on these results and those of others, it is recommended that fish culturists exercise juvenile salmonid fish regularly, especially those to be released into the wild. If the fish are being reared for market rather than release, it would appear that faster growth and better food conversion can result from such a regimen. If enough water is not available to create the necessary water velocity in typical raceways, then circulating rearing tanks such as Swedish or circular ponds can be used.

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A Comparison of Four Apparatuses for Recovering Infectious Pancreatic Necrosis Virus from Rainbow Trout

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Abstract.—Four tissue dispersion apparatuses were compared to determine which was most effective in recovering infectious pancreatic necrosis virus (IPNV) from rainbow trout (*Salmo gairdneri*). The Omnitron, Polytron, Stomacher, and mortar and pestle, routinely used in fish virology laboratories, were equally effective in liberating IPNV from carrier rainbow trout tissues. Viruses were found in 50–52.5% of the fish with each apparatus and there were no significant ($P = 0.05$) differences in the concentrations of virus recovered by the four methods. The results indicated that several apparatuses are suitable for recovering IPNV from trout tissues. The advantages and disadvantages of each piece of equipment are discussed.

Several apparatuses are available for liberating viruses from tissues of infected animals. The most commonly used devices in fish virology laboratories disperse tissues by grinding or blending, with or without sonication (Wolf 1970; Hill 1976). Reports comparing the effectiveness of these apparatuses for detecting viruses in fish tissues are lacking. The purpose of this study was to compare four apparatuses, now in routine use in fish health laboratories, to determine which is most effective in recovering infectious pancreatic necrosis virus (IPNV) from tissues of carrier rainbow trout (*Salmo gairdneri*).

The IPNV is found in many areas of the world where trout are reared (Pilcher and Fryer 1980) and similar viruses have also been isolated from several nonsalmonid fish (Adair and Ferguson 1981). As a result, fish infected with IPNV are encountered in many virology laboratories. Trout that survive IPNV infections as younger fish become lifetime carriers, with virus replication continuing in the spleen, kidney, pyloric-ceca, intestine, and gonads (Billi and Wolf 1969; Yamamoto and Kilistoff 1979). The shedding of infectious viruses from carriers and the stability of this virus in the environment have contributed to the spread of IPNV, via fish and egg shipments, to most major trout producing areas in the world (Pilcher and Fryer 1980).

The IPNV can tolerate considerable changes in pH and temperature without loss of infectivity (Mackelvie and Desautels 1975). In addition, approximately 50% of the viruses stay associated with cells in tissue culture (Malsberger and Cerini 1963). These properties, and the availability of large carrier fish, made IPNV suitable for comparing the efficiency of four apparatuses commonly used for extracting viruses from tissues.

Methods

Forty rainbow trout from a hatchery population known to contain fish persistently infected with IPNV were placed on ice and transported to the laboratory. The fish were 9 months old (from hatching date) and averaged 90.9 g in weight.

Tissue Preparation

The entire kidney and spleen and portions of the pyloric-ceca from individual fish were aseptically removed and placed on a petri dish. Each organ was then briefly mixed with forceps, divided into four approximately equal portions, and distributed to tared petri dishes. The weights of the four portions containing kidney, spleen, and pyloric-ceca were adjusted to within 0.1 g and then refrigerated at 4°C.

Prior to dispersion, the tissues were suspended (1:40 weight: volume) in cold minimal essential medium (MEM, Flow Laboratories) without serum, glutamine, or antibiotics. The medium was buffered at pH 7.2 with *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES, United States Biochemical Company).

Tissue Dispersion

The four apparatuses designed to facilitate the release of virus from tissues were: (1) mortar and pestle, (2) Stomacher 80 (Colwell, Incorporated), (3) Polytron (Brinkman Instruments), and (4) Omnimixer (Sorvall, Dupont Company).

Sterilized mortars and pestles were chilled at 4°C prior to use. Four grams of Alundum (90 mesh, Matheson-Coleman-Bell Company) placed in the mortar was used as a grinding matrix for the tissue suspension. Tissue dispersion was accomplished by 50 circular motions of the pestle.

The Stomacher 80, employed in certain food microbiological studies (Jay 1979), utilizes the shearing motion generated by two opposing paddles against a steel plate. The tissue suspension, locked in a sealed plastic bag between the paddles and plate, was dispersed for 2 min without cooling.

The Polytron uses a combination sonicating and grinding tip to disperse the tissue. The tip (PT10) was immersed directly into a centrifuge tube containing the tissue and diluent. Homogenization continued for 10 s at setting number 6, without cooling. The Polytron tip was disinfected between samples by a 10-s treatment with 200-ppm chlorine followed by two 10-s rinses with distilled deionized water.

The Omnimixer, designed to shear tissues by means of rapidly rotating blades in a sealed stainless steel cup, was also used to extract virus from tissues. The tissue suspension was placed directly into the chilled stainless steel cups. The tissues were homogenized for 2 min at high speed (100,000 revolutions per minute); the cups were cooled in an ice bath.

The homogenates from all four tissue preparation methods were transferred to centrifuge tubes and held at 4°C. Cellular debris was removed from all samples by centrifugation at 2,600 × gravity for 20 min at 4°C. After centrifugation, a 1.0-mL aliquot of tissue homogenate was transferred to a tube containing 1.5 mL of MEM fortified with penicillin 2,000 International Units and streptomycin 2,000 μg/mL (Gibco, Incorporated), gentamycin 1,000 μg/mL (Schering Company), and Fungizone 50 μg/mL (Gibco, Incorporated) to control microbial contamination (Amend and Pietsch 1972). The mixture was incubated overnight at 4°C and then centrifuged at 2,600 × gravity for 20 min prior to virus titrations.

TABLE 1.—Concentrations (plaque-forming units per gram of tissue) of infectious pancreatic necrosis virus (IPNV) detected in rainbow trout tissues by four apparatuses for tissue dispersion. Empty data cells indicate the virus was not detected in the lowest assay dilution.

Fish number	Fish weight (g)	Dispersion apparatus			
		Omnitron	Polytron	Stomacher	Mortar and pestle
1	77.5	7,500	2,000	14,000	8,000
2	131.1	4,000	43,000		500
3	105.4	25,000	60,000	180,000	15,000
4	96.2	2,000		1,000	1,500
5	101.0				
6	105.9				
7	104.9				1,000
8	109.9		500		
9	135.8		500	3,000	1,000
10	92.9				
11	115.6				
12	70.4				
13	120.4	42,000	20,000	47,000	40,000
14	109.4	3,000	18,000	5,000	7,000
15	81.0				
16	45.7	15,000	22,000	80,000	71,000
17	107.7	25,000	42,500	25,000	26,000
18	95.6			48,000	10,000
19	87.0				
20	104.6			10,000	
21	82.5	5,000			
22	97.7	29,000	23,000	20,000	24,000
23	109.8	2,000		1,000	
24	96.2	1,000	3,000	13,500	1,000
25	146.9	10,000	2,000	2,000	6,000
26	83.2	500	4,000	46,000	1,000
27	109.1				
28	99.8	8,000	21,000	20,000	25,000
29	55.3	27,000			
30	92.3	4,000	2,000	2,000	1,000
31	85.7				
32	112.5		500	1,000	500
33	47.6				
34	99.5	1,000	1,000	500	
35	61.0				
36	55.8				
37	50.6	90,000	80,000	110,000	38,500
38	53.0		1,000		
39	50.8	24,000	45,000	57,500	30,000
40	49.2				
Total positives		20	20	21	20

Virus Titration

Monolayer cultures of CHSE-214 cells, a line derived from chinook salmon (*Oncorhynchus tshawytscha*) embryos, were prepared in 24-well dishes (Linbro Company) and inoculated so that undiluted and serial tenfold dilutions of the virus suspension (0.05 mL) were distributed to each of

TABLE 2.—Friedman two-way analysis of variance comparing four apparatuses for tissue dispersion for the recovery of infectious pancreatic necrosis virus (IPNV) from carrier rainbow trout.

		Dispersion apparatus					
Statistic	Number of fish	Omnitron	Polytron	Stomacher	Mortar and pestle	$\chi_r^2 = 0.05$	Critical value χ^2
r_j[a]	40	96.5	99.5	109.5	95.0	1.78	7.82
r_j[b]	14	30.0	35.0	42.5	32.5	3.72	7.82

[a] Comparison includes all fish examined.

[b] Comparison includes only those fish from which IPNV was recovered by all four methods; df = 3.

two replicate wells. After a 30-min adsorption period, the residual inoculum was removed, and a semi-solid overlay containing equal parts of 1.6% agarose (Seakem, Incorporated) and double-strength MEM (pH 7.2) with 5% fetal bovine serum (Sterile Systems, Incorporated) was added (Wolf and Quimby 1973). The plates were chilled at 4°C for 30 min and then placed at 20°C.

After 3 d incubation, the cells were fixed with 10% neutral buffered formalin for 1 h. The agar was then removed, and the cells were stained with 1% crystal violet in 95% ethanol. The numbers of plaques counted in duplicate wells were then averaged and were expressed as plaque forming units (PFU) per gram of original tissue. Confirmation of IPNV infection was made by picking plaques from selected cell monolayers prior to fixation. A suspension made from the plaques in MEM was then tested with hyperimmune rabbit serum to IPNV (Strain VR299 IPNV) in a standard neutralization test (Okamoto et al. 1983).

Results

All four methods of tissue dispersion resulted in detection of IPNV in selected rainbow trout tissues (Table 1). Because of the dilutions required to homogenize tissues, the final inoculum was 1:200 weight : volume. The lower limit of detection, given an 0.05 mL inoculation, at this dilution was 500 PFU/g. Many fish were at or fell below this limit while others contained up to 1.8×10^5 PFU/g.

The four methods were equally effective in recovering viruses from infected fish (50 to 52.5% detection rate). Viruses were not found by any technique in 13 fish. Of the remaining 27 fish, 13 were determined to be positive by at least one method but not by all four; 10 of these fish had virus concentrations at or near the limit of detection.

A Friedman two-way analysis of variance was used to compare the four methods. This analysis showed no significant differences (critical value $P =$ 0.05) among methods whether all 40 fish or just those 14 fish positive by all four methods were considered (Table 2).

Discussion

Procedures for detecting IPNV by cocultivation of kidney cells from carrier trout with susceptible fish cell lines have been described (Agius et al. 1982). Although cocultivation may be possible under certain conditions, most fish health laboratories homogenize tissues in one of several apparatuses prior to cell inoculation (McDaniel 1979). In this study, four of the most commonly used devices were found to be equally effective in recovering IPNV from infected trout. The concentrations of virus detected in trout in this study were similar to those previously reported from carrier rainbow trout (Reno et al. 1978) and brook trout (*Salvelinus fontinalis*) (Billi and Wolf 1969; Reno et al. 1978; Yamamoto and Kilistoff 1979; Hedrick and Fryer 1982). Concentrations of virus recovered from certain fish varied substantially among techniques. We attributed this to differences in the distribution of the virus in the tissues. It has been shown by indirect fluorescent antibody tests that viral antigens (presumably infectious viruses) are not distributed evenly in the tissues of infected fish. Instead they appear as distinct focal areas (Swanson and Gillespie 1981). We tried to minimize variations due to this uneven virus distribution by gently mixing the excised tissues prior to division for dispersion with each of the four apparatuses. In most cases, virus recoveries from a fish varied little among techniques. Exceptions were fishes 2, 3, 18, 26, and 29 (Table 1).

Approximately 50% of the fish examined by each technique were determined to be infected, although many fish were at, or near, the limits of detection (500 PFU/g of tissue; Table 1). A nonparametric statistical analysis indicated no significant differences among tissue dispersion methods at the $P = 0.05$ level (Table 2).

These results indicate that laboratories involved in virological studies can choose among at least four apparatuses for adequate recovery of IPNV from fish tissues. We preferred the Stomacher for convenience because its closed system (sealed bags) prevents the aerosol formation characteristic of the Polytron and mortar and pestle. In addition, the plastic bags are disposable, so rewashing and resterilization of grinding vessels are not required as they are with the Omnitron and mortar and pestle. Samples collected in the field may also be placed directly into the Stomacher bags, which may be obtained presterilized. In contrast to our results, Agius et al. (1983) found that the Stomacher was less efficient than either ultrasonic treatment or the mortar and pestle in recovering IPNV from kidney tissues. However, as those authors stated, their results must be considered preliminary because small groups of fish ($N = 6$) were used in the comparison. Furthermore, in their study they did not titrate virus concentrations nor were tissues from the same fish used to compare the apparatuses.

Both the Polytron and Omnitron were more efficient in reducing hard and soft tissues to a homogenous suspension than the Stomacher. This, however, had no evident effect on the concentrations of IPNV that were subsequently recovered. The Polytron required only 10 sec for complete blending compared to the 2 or more minutes for the other apparatuses. Additional time was saved by homogenizing the sample directly in the centrifuge tube which eliminated washing and sterilization of equipment. However, considerable aerosol formation was observed with the Polytron. This and the effect of sonication on viral infectivity, especially with viruses less resistant to physical changes, were viewed as the main disadvantages of the Polytron. Incomplete tip disinfection between samples, which might pose a problem with the Polytron, was not detected, as sequentially processed samples did not indicate virus carry-over (Table 1).

The Omnitron mixer, which also gave complete blending, had closed cups that prevented aerosol formation. However, both the washing and resterilization of the stainless steel cups and the adjustments required to keep the blades running smoothly were time consuming.

The mortar and pestle was by far the least expensive apparatus. Aerosol formation was observed and, again, washing and resterilization made it less convenient.

The results of these studies indicate that at least four apparatuses are satisfactory for the recovery of IPNV from carrier rainbow trout tissues. Each apparatus has advantages and disadvantages that should be considered when decisions are made about which equipment to use in a fish health laboratory.

Acknowledgments

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COMMUNICATIONS

Altering the Spawning Cycle of Rainbow Trout by Manipulating Water Temperature

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It is often desirable to provide eggs or fry from a particular strain of rainbow trout (*Salmo gairdneri*) over an extended period of time. Availability can be prolonged by incubating eggs in cold water and thus slowing embryonic development. However, an experiment conducted at the Bozeman Fish Cultural Development Center in which rainbow trout eggs were exposed to temperatures of 13, 10, 6, and 3°C (55.5, 50, 43, 37.5°F) showed a significant reduction in the percentage of eyed eggs and the survivability of eggs held at the lowest temperatures (unpublished data). Published studies on egg incubation of various salmonid species indicate that temperatures below 4.5°C (40°F) are detrimental (Davis 1953; Combs and Burrows 1957; Combs 1965; Hokanson et al. 1973; Leitritz and Lewis 1976). Another possible method of extending egg availability is to hold broodstock in cold water, and thus delay egg and sperm development. The present study was designed to compare spawning time and egg quality of rainbow trout broodstock maintained in cold creek water with those of brood stock maintained in constant temperature spring water.

The facilities at the Bozeman Center offer the opportunity to use Bridger Creek or spring water in the raceways. Chemical characteristics of the water supplies are similar. Alkalinity and total hardness (both in mg/L as $CaCO_3$) and pH of the creek water were 189, 203 and 8.2, respectively (Russo and Thurston 1974); values for spring water were 181, 207 and 7.6 (analysis by Water Chemistry Laboratory, Montana State University, Bozeman, Montana).

Methods

Two hundred fifty, 3-year-old, Winthrop strain rainbow trout were held in each of two outdoor raceways measuring 1.8 × 18 × 0.6 m (6 × 60 × 2 ft). The trout had been reared in spring water and the raceways were initially provided with this water. In early September, creek water was introduced into one raceway; the other continued to receive spring water at 10 ± 1°C (50 ± 1°F). Water inflow for each raceway was approximately 570 L/min (150 gal/min). Fish were checked weekly beginning in December and artificially spawned by manual stripping when ripe. The initial spawn from each group was discarded; egg quality was believed to be poor because optimum spawning time was missed.

Fish were anesthetized in a 1% salt solution containing 50 mg/L of MS 222 and then checked for ripeness. Ripe females were rinsed with fresh water and the eggs spawned into a strainer to drain off excess fluid. Eggs were then placed in a pan and fertilized. On each spawning day, nine to 18 females were spawned separately and fertilized with sperm from separate males. If additional females were spawned, eggs from three fish were pooled and fertilized with sperm from two males. Eggs were incubated in individual and pooled lots in Heath incubator trays supplied with spring water at the rate of 19 L/min (5 gal/min). Eggs were treated daily with formalin at a concentration of 1:600 for 15 min to control fungus. When eyed, eggs were counted with a Veeder-Root electronic

Table 1.—Egg production of rainbow trout broodstock held in spring water at 10°C (50°F) and Bridger Creek water at lower temperatures.

Water source	Weight of female (kg)	Fecundity: Eggs per female	Fecundity: Eggs per kg/female	Weight per 1,000 eggs (g)[a]	Eyed eggs (%)
Spring	1.99[b]	2,992	1,527	75	86
Creek	1.75	3,094	1,793[b]	72	86

[a] Data for all fish, pooled.

[b] Value significantly higher ($P < 0.05$) than the other value in the same column.

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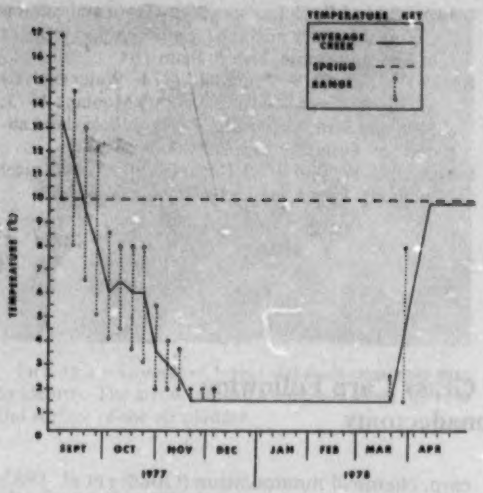

FIGURE 1.—Average temperature and ranges for creek versus spring water, beginning September 6, 1977 (Week 1).

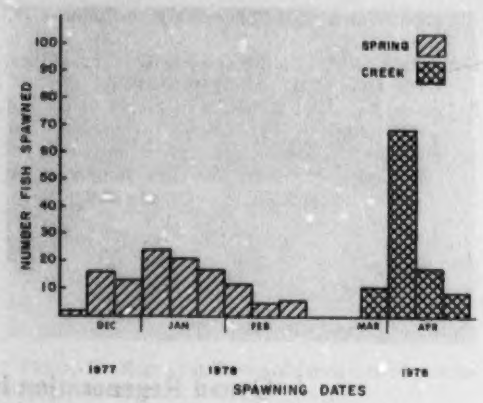

FIGURE 2.—Number of rainbow trout held in spring water or creek water, spawned on different dates.

counter. Dead eggs were picked out by hand with a suction bulb and glass tube.

Data collected for individual fish included female weight, total number of eggs per female, number of eggs per kilogram of body weight, and percent of eyed eggs (Table 1). A comparison of two samples test (*t*-test) was used to statistically analyze the data (Snedecor and Cochran 1967).

Results

Average weekly temperatures of the creek water ranged from 2°C (35°F) to 14°C (57°F) (Figure 1). Fish held in spring water were spawned from December 12, 1977, to February 9, 1978, and fish held in creek water from March 29 to April 20, 1978 (Figure 2). When fish held in creek water were ready to spawn, water temperature was about 7°C (45°F) and rising rapidly. At this time, the creek water became heavily silted and was replaced with spring water.

A total of 39 females were spawned from the creek water and 66 females from the spring water. Gross observations such as "blood in eggs" or "some bad eggs" were made when spawn was taken. Fish observed with these conditions were not considered in the individual comparisons, because these eggs would normally be discarded. However, the data were included when overall egg weight per thousand eyed eggs was calculated (Table 1).

Fish held in spring water were significantly larger at spawning time than those held in creek water; a difference directly related to increased food intake and higher metabolism at the higher water temperatures. Fish held in creek water produced more eggs per kilogram of body weight than did those in spring water, but the eggs were smaller; consequently, there was no significant difference in weight of eggs per kilogram of fish between groups. Neither was there a significant difference in total number of eggs per female or in percent eyed eggs between the fish held in spring water and those held in creek water (Table 1).

This study suggests that, if late-season rainbow trout eggs are desired, broodfish (at least of the Winthrop strain, and perhaps other late fall and winter strains) can be held in cold water to delay spawning without affecting egg quality. We also demonstrated that a domestic hatchery strain of fish that spawns in early winter in a hatchery environment can revert to typical spring spawning when exposed to temperatures experienced in the wild.

Acknowledgment

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Gonad Regeneration in Grass Carp Following Bilateral Gonadectomy

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Grass carp (*Ctenopharyngodon idella*) are an efficient means of controlling aquatic macrophytes, especially *Hydrilla verticillata* (Shireman and Maceina 1981). However, the threat of this exotic fish reproducing in natural waters has caused concern (Conner et al. 1980). Methods are needed to render grass carp sterile yet maintain their efficacy. Techniques that have received the most attention from researchers have involved hybridization of female grass carp and male bighead carp (*Hypophthalmichthys nobilis*) (Marian and Krasznai 1978; Beck and Biggers 1983), induction of triploidy in grass carp, chemical manipulation (Goudie et al. 1983; Jensen et al. 1983), and gonadectomy. The latter was thought to be a valid and effective means for maintaining the voracious feeding habits of grass carp while eliminating the threat of natural spawning.

Before considering a program utilizing gonadectomized fish, a test study was initiated to resolve whether grass carp gonads would regenerate and be functional. Several researchers had indicated that regeneration could occur in grass carp, but no determination was made on the ability of the regenerated organs to function in reproduction (Wunder 1955; Sonneman 1971; Brown and Richards 1979). Robertson (1961) did find that kokanee salmon (*Oncorhynchus nerka*) regenerated testicular tissue in the posterior region adjoining the spermatic duct, resulting in spermatogenesis. He

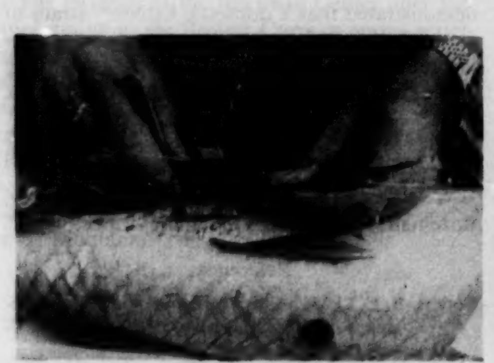

FIGURE 1.—Incising of ventral surface just anterior to the vent. Incision is long enough to permit complete removal of gonads.

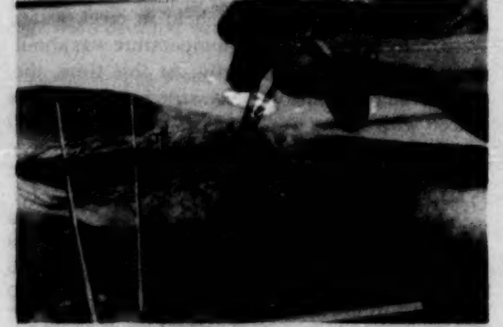

FIGURE 2.—Removal of a testis from a grass carp. The gonad is stretched and scissors inserted posteriorly as far as possible before the organ is cut.

FIGURE 3.—Gonads of 5-year-old grass carp were easy to identify. The arrow indicates the testis along the ventral surface of the air bladder.

FIGURE 5.—Regenerated testis of a grass carp 10 months after gonadectomy.

also found that females would regenerate ovarian tissue which eventually developed a limited number of eggs.

Methods

In August 1983, the gonads of 26, 5-year-old grass carp (mean weight of 1,749.8 g) and of an additional 13 grass carp (1–3 years old) were removed. The procedure involved making an incision just anterior to the vent (Figure 1). Gonads were extracted by cutting the extreme anterior and posterior points of attachment (Figure 2) and removing the entire organ, along with surrounding mesentary. The mesentary was removed, since Brown and Richards (1979) and Sonneman (1971) felt any regeneration that occurred resulted from tissue that had been close to the removed gonad. This was supported by the regrowth found in grass carp from which only one-third of each gonad had been removed and mesentary left behind; the gonads showed some regeneration in all of the mature gonadectomized grass carp and in 96.7% of the immature 1–2-year-old fish (Clippinger and Osborne 1984). Gonad excision in the present study was relatively simple, since ovary and testis were large enough to easily identify and remove from anaesthetized (15 ppm quinaldine) fish (Figure 3). Nitrofurazone (9.3% active) was sprinkled into the incision and "simple interrupted" sutures of 3-0 silk were used to close it (Figure 4). Fish were returned to vegetated ponds.

Results

In June 1984, 37 gonadectomized fish were recovered and examined for regeneration (mean weight 4,184.7 g). Twenty-three were males, 21 of which had regenerated testes and were producing

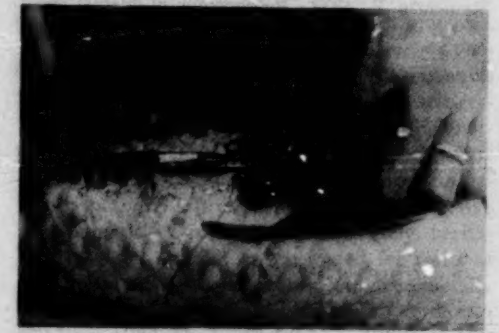

FIGURE 4.—The incision was closed with "simple interrupted" sutures of 3-0 silk. Sutures were spaced every 2–3 cm.

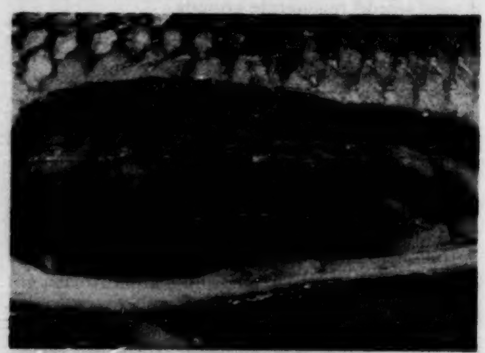

FIGURE 6.—Incomplete regeneration of an ovary (arrow) in a gonadectomized female grass carp.

seminal fluid (Figure 5). These fish were potentially able to reproduce, since seminal fluid flowed from the vent when palpated. One male grass carp was found with testes producing fluid, but there was no connection via a duct to the urogenital pore; therefore, the fluid could not be released. Four females were considered capable of reproducing because their soft, distended abdomens contained eggs and, when stripped, several ova were expelled through the oviduct. Ovulation was induced in one of these females with a series of human chorionic gonadotropin and carp pituitary injections. This female yielded approximately 360,000 eggs, which were fertilized and subjected to thermal shocks to induce triploidy. The process produced 6,800 viable fry (both diploid and triploid), which were stocked into hatchery ponds. The three remaining females were held for future use. Another female also showed complete regeneration, but no advanced-stage ova were found. Eight females exhibited incomplete regeneration of the ovaries, which were in a stage of development comparable to younger fish (Figure 6). These fish were also missing an oviduct on either one or both ovaries. On ovaries that were not connected to the urogenital pore by an oviduct, an appendage originated where an oviduct should be located. This appendage appeared to be a developing oviduct, and had the same color and texture as a functional duct. Only two fish were observed that did not have identifiable gonad regeneration; these were 1-year-old male and female carp. In the study conducted by Clippinger and Osborne (1984), only 65.5% of the immature males and 57.4% of the immature females that were given gonadectomies had regenerated enough tissue after 7 months to produce open pathways for gametes. Further evaluation of young fish would be needed to determine if they would regenerate gonads.

Similar results have been found by personnel of the Coachella Valley Water District, California (personal communication, Dr. Paul Beatty). They have ceased using gonadectomized grass carp because of their findings, and because of the recent availability of triploid grass carp. Based on the above results, gonadectomies should be considered an ineffective procedure for rendering grass carp incapable of reproducing.

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Selective Breeding and Line Crossing to Reduce Inbreeding

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Abstract.—Selective breeding is a complex problem. A simple procedure which involves two separate lines and line crossing every third generation is presented. The advantages are increased genetic performance and reduced rate of inbreeding. The procedure and theory are discussed. Particular emphasis is placed on the need to avoid inbreeding, which can lead to loss of the breeding population.

Improving stock performance is an important priority for the majority of aquaculturalists. While the importance of continually refining husbandry practices cannot be overemphasized, selective breeding is often ignored. Breeding associations, private corporations, and government agencies actively design, promote, and support genetic improvement programs with increased productivity as the ultimate goal in domestic animals. A comparison of the current production levels of most domestic animal breeds with those from 50 to 100 years ago illustrates that a combination of directed broodstock selection and sound husbandry can result in substantial benefit to the producer.

The physical appearance or expression of a trait in an individual is known as its *phenotype*. This results from its *genotype*, the *environment* in which it lives, and the *interaction* between genotype and environment. An individual with an outstanding genotype may have a poor phenotype if, for some reason, the environment masks the expression of the genotype. In other words, a superior genotype is no protection against poor husbandry practices.

The ability of an individual to transmit its phenotype to the next generation can be viewed as heritability (Jacquard 1983). While this is an oversimplification of the heritability concept, high heritability values indicate large amounts of genetic variation. Zero heritability indicates that no genetic variability is present, and therefore, selective breeding will be ineffective. Fortunately, heritabilities which have been estimated for traits of economic importance in salmonids have generally been greater than zero (Gjedrem 1983; Gjerde and Gjedrem 1984; Hurley and Schom 1984).

Genetic variation exists when more than one form of a gene is present, and different forms of the same gene are known as alleles. The objective of selective breeding is to increase the proportion of favorable alleles. However, the actual genotypes of individuals are unknown, and can only be inferred from their phenotype. By selecting superior parents only, the proportion of favorable alleles will be increased in the next generation. This means that the average performance of individuals in the next generation will be higher than the average of the parent generation. To make this gain in performance, the traits that are to be improved must be measurable on some repeatable scale. In this way, the genetically superior animals in the population can be accurately identified. The heritability of all traits to be improved must be greater than zero. In addition, there must be more broodstock than is required for production, and the level of inbreeding must be kept to a minimum.

No single approach to selective breeding is universally best. Many breeding schemes require expensive facilities, are labor intensive, and are not suitable for the average producer. Furthermore, many protocols promising rapid genetic gains may promote inbreeding with the long-term risk of reduced performance. The effects of inbreeding have been widely discussed (Fisher 1949; Falconer 1981) and demonstrated in several salmonid species (Ryman 1970; Kincaid 1976a, 1976b).

If all the broodstock are required for production, there can be no genetic selection program. Also, a program designed to increase the proportion of favorable alleles in a population carries inherent risks because some unfavorable alleles will also be increased. As generations pass, the continued accumulation of unfavorable alleles may lead to reduced performance in the population. It is a problem that develops when relatives are mated. The closer the relationship, the greater the problem, because relatives carry some of the same unfavorable alleles transmitted to them from their common ancestor. These can combine, producing the homozygous state of the allele in their offspring. The net effect (reduced performance) is called inbreeding depression and is an explanation for performance falling below expectation (Gjedrem 1983). The depression occurs at a rate of about 4.5% per 10% inbreeding (Gjerde et al. 1983). Inbreeding alone is not necessarily bad, since combining favorable alleles is the goal of selection. It

TABLE 1.—Levels of inbreeding in a closed population after 1, 2 and 10 generations of random mating for various numbers of parents.

	Number of breeding individuals							
Generation	106	80	40	30	25	16	5	2
1	0.005	0.006	0.013	0.017	0.020	0.031	0.100	0.250
2	0.009	0.012	0.025	0.033	0.0396	0.062	0.190	0.438
10	0.050	0.067	0.124	0.169	0.183	0.294	0.651	0.944

is the increased proportion of the homozygous unfavorable alleles in the breeding population that leads to problems.

In closed populations, the level of inbreeding accumulates from one generation to the next. The rate of increase depends on the effective number of parents that contribute to the next generation. The effective population size represents a weighted average of the number of males and females that contribute progeny (Table 1). Inbreeding can be dramatically reduced if a minimum of 40–50 individuals of the least numerous sex are chosen at random as broodstock in each generation.

An inexpensive selection and broodstock management procedure is proposed here that can produce both genetic gain and offer long-term protection against inbreeding. The protocol requires fewer separate lines than the procedure outlined by Kincaid (1977), plus has the advantage of ridding the lines of unfavorable alleles (McNeil et al. 1984b).

Procedure

To implement the selective breeding procedure, there must be facilities available to maintain two separate lines for a complete generation. Although one of the two groups can be marked, either by clipping the adipose fin or branding, and combined in a common tank or pond, it would be preferable for the lines to be maintained separately at all times. To establish the two lines, a minimum of 40–50 single paired matings of unrelated broodstock should be made for each line. No fewer than 40 mated pairs should be used, and a larger number would be preferable, if facilities are available (Kincaid 1983). If the pedigree of the fish is unknown, it would be advisable to select only half of the broodstock requirements from that stock and obtain a similar number from an unrelated, second source. Each mating pair should then consist of one fish from each source. By crossing the fish from two unrelated sources you will ensure that the level of inbreeding in the initial population is zero, and minimize the danger of inbreeding depression. Once the matings have been made, randomly assign eggs from each pair to the different lines. This is the parental generation or generation zero.

For the next two generations, mate within lines only (e.g., mate line A males with line A females and line B males with line B females). In the F_1 and F_2 generations, the broodstock should be selected on the basis of individual performance of traits considered desirable, such as good survival, fast growth, and conformation. This is known as mass selection; the best fish are chosen from the mass (Falconer 1981).

In many hatcheries, milt from more than one male is used to reduce the possibility that an infertile male will ruin a spawning of fertile eggs. If single matings cannot be made, the milt from several males should be combined and thoroughly mixed before it is added to the eggs. This will minimize the chance that the first male used will fertilize the bulk of the eggs.

The F_3 generation is produced by selecting males and females by the same mass selection procedure used in generations 1 and 2. However, the lines are crossed by mating the males from line A to the females from line B and males from line B with females from line A. This will produce two line crosses (A males × B females and B males × A females), reducing the accumulated level of inbreeding to zero in the initial cycle (MacNeil et al. 1984b).

The procedure recycles following the F_3 generation. The cycle can be continued indefinitely (Figure 1). If the fish take 3 years to mature, then there will be three separate age groups of each line. Each brood fish should be spawned only once. Using the same fish for several different years reduces the effective number of parents and increases the rate of inbreeding.

Summary

The approach described is a modification of procedures used to reduce the effect of inbreeding depression in Japanese quail (*Coturnix coturnix*

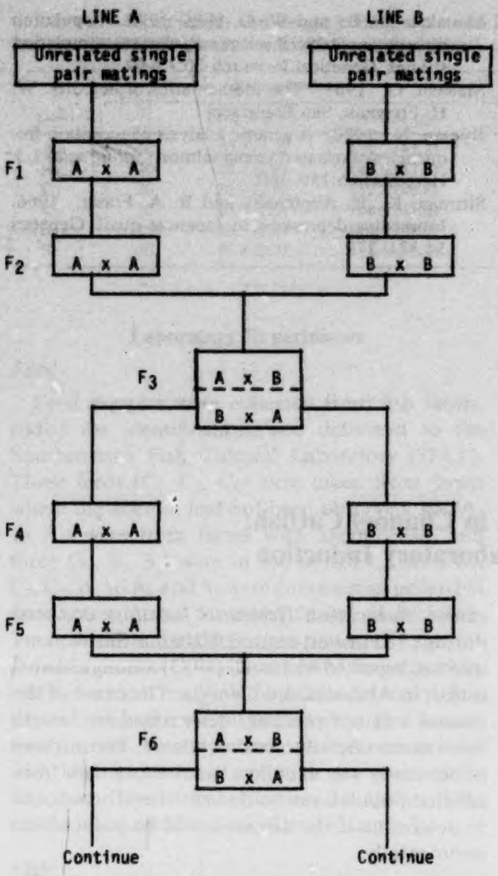

FIGURE 1.—Selective breeding procedures, combining mass selection with line crosses to minimize level of inbreeding while maximizing the net genetic gain.

japonica) (Sittman et al. 1966; MacNeil et al. 1984b) and the ones used in a computer simulation experiment designed to estimate the relative efficiency of selection and breeding procedures (Madalena and Hill 1972). The authors report that gradual increases in the level of inbreeding allow for the elimination of deleterious recessive genes, (i.e., individuals expressing sub-viable homozygous genotypes will be eliminated from the breeding program because of their poor performance). These alleles will, however, not be completely purged from the population, at least in the early generations. Specifically, the level of inbreeding increases during the first two generations of mating within lines (Table 1), but then drops to zero when the lines are first crossed (Table 2). Using a variation of this scheme, MacNeil et al. (1984a, 1984b) were able to keep inbreeding below 1% through 17 generations.

TABLE 2.—Rate of inbreeding based on the smallest possible number of parents (2), and the number (80) recommended to start the program, calculated by procedures of Malécot (1969). The real situation would be somewhere in between. Illustrated is the kind of drop in inbreeding that can be expected when crosses are made between the two inbred lines at generation 3 and 6. The rate of increase is much smaller with a larger number of parents. See Table 1 for expected results through the first two generations with differing numbers of parents.

	40 of each sex		1 of each sex	
Generation	Line A	Line B	Line A	Line B
0	0	0	0	0
1	0.006	0.006	0.25	0.25
2	0.012	0.012	0.44	0.44
3	0	0	0	0
4			0.34	0.34
5			0.42	0.42
6			0.25	0.25

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Diet Related Anemia in Channel Catfish: Case History and Laboratory Induction

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Abstract.—Severe anemia among fingerling to adult channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*) was reported by 39 of 166 catfish farmers in west-central Alabama in 1983. The hematocrits of moribund fish in ponds ranged from 0 to 5%. Weekly mortalities as high as 5% in individual ponds were recorded. The gills of affected fish were pale or white, the kidneys and spleen were pink, and the liver was gray to tan. In 27 of 29 ponds where the type or brand of feed was changed, deaths ceased within 1 week. However, the disease was noted among fish regardless of the major brand or type of feed used. Under laboratory conditions, the feeding of diets from affected farms induced anemia in fry, but not in subadults. The cause of the anemia was associated with an unidentified factor or factors in diets.

Severe anemia among certain lots of farm-raised channel catfish (*Ictalurus punctatus*) and to a lesser extent blue catfish (*Ictalurus furcatus*) occurred during 1983 in west-central Alabama. Similar anemia was reported by Lovell (1983) among channel catfish in Alabama and Georgia. The cause of the disease was not precisely determined by Lovell, but was considered to be diet related. The purpose of our study was to collect case history data from affected populations, to describe the disease, and to determine if the disease could be induced experimentally.

Methods

During necropsy of moribund fish from commercial ponds, blood was collected for hematocrit determination; gills, skin, and internal organs were observed grossly for lesions; and the fish were examined microscopically for parasites. The liver, posterior kidney, and brain were streaked on brain-heart infusion medium, and any bacterial growth was presumptively identified by using the biochemical procedures of Plumb and Bowser (1983). Selected feed samples collected from farms with anemic fish were analyzed for lead, aflatoxin, zearalenone, and insecticides at the Alabama Veterinary Diagnostic Laboratory, Auburn, Alabama. Carbon dioxide, nitrite, and total ammonia nitrogen in pond water were determined with a Hach Kit. Alkalinity was determined in accordance with procedures described by the American Public Health Association et al. (1980). Dissolved oxygen was determined with an oxygen meter and hydrogen ion concentration with a pH meter.

TABLE 1.—Mean length and weight before, and mean length, weight, and hematocrit after 97 d of experimental feeding of subadult channel catfish (SD in parentheses).

	Before feeding			After feeding for 97 d			
Diet	*N*	Length (cm)	Weight (g)	*N*	Length (cm)	Weight (g)	Hematocrit (%)
A_6	20	20.2 (2.2)	131.2 (42.3)	17	23.7 (2.8)	236.2 (74.9)	38.8 (4.4)
A_6	20	20.7 (1.9)	142.9 (43.8)	20	26.0 (2.4)	337.1 (115.0)	36.5 (4.7)
C_3	20	20.9 (2.0)	152.8 (46.8)	20	27.2 (2.9)	381.5 (128.6)	37.2 (3.5)
C_3	20	20.9 (2.0)	168.3 (47.8)	20	27.7 (2.5)	399.1 (111.6)	41.1 (3.7)
S_3	20	20.5 (2.2)	161.7 (48.9)	20	28.4 (2.4)	435.4 (124.1)	40.8 (3.3)
S_3	20	19.8 (2.2)	143.6 (49.7)	19	26.9 (2.9)	360.7 (126.0)	41.3 (3.0)

Laboratory Experiments

Feed

Feed samples were collected from fish farms, coded for identification, and delivered to the Southeastern Fish Cultural Laboratory (SFCL). Three feeds (C_1, C_2, C_3) were taken from farms where the anemia had not been observed, six (A_1 to A_6) were from farms with anemic fish, and three (S_1, S_2, S_3) were in use at SFCL. Diets C_1, C_2, C_3, A_1 to A_6 and S_3 were commercial pelletized rations formulated for subadult and adult catfish. There were no fry diets in use on farms reporting the anemia. Diet S_1 was formulated for salmon fry and S_2 for trout fry. Pelletized diets fed to fry were ground until all material passed through a 1-mm-mesh screen. A sample from diet A_6 (associated with high incidence of anemia) was analyzed by Woodson Tenent Laboratories, Memphis, Tennessee, for pantothenic acid and vitamin E.

Fish

Subadult channel catfish were obtained from a pool of unaffected fish at SFCL that had been maintained on diet S_3 in aerated well water at 20°C. Fish were anesthetized, weighed, and measured; 20 fish were introduced into each of six 190-L tanks supplied with aerated well water at 20°C (Table 1). Mean hematocrit for a sample of 10 pooled fish was 35.9 ± 2.9% (SD). All fish were fed diet S_3 for 2 weeks before being fed the test diets. Fish in each of two tanks were fed diets A_6, C_3, or S_3 at 3% of body weight per day for 14 weeks (Table 1). At 2-week intervals, all fish in each tank were anesthetized and the gills examined for evidence of anemia. On day 97, all fish were weighed and measured, and hematocrit values determined.

Another group of fry from different lots at SFCL was pooled and maintained in pond water at 23°C. A group of these fry were acclimated to 20°C and divided into two tanks. After 24 h, 500 fry (9.7 fish per gram) were placed into each of 11, 50-L tanks supplied with aerated well water at 20°C and fed diet S_2 for 2 weeks before being fed the test diets. The fry were fed diets C_2, C_3, A_1–A_6, and S_1 and S_2 6 times per day for 10 weeks; at 2-week intervals, variable numbers were examined for evidence of anemia (Table 2). Fry fed diet C_1 did not survive beyond 16 days. Fry with white gills, light pink to colorless blood moving through the heart, and few blood cells moving through the caudal veins were classified as anemic. All fish in each tank were examined at the final sampling for evidence of anemia. In addition, on the final day of the study, a subsample of fry from each tank was counted and weighed to determine average weight and a blood sample was collected for hematocrit determination.

Results

Case History

The anemic condition was observed in fish from 39 of 166 catfish farms in west-central Alabama, and was associated with diet, not bacteria, parasites, or water chemistry. Mortality related to anemia began in April, became more prevalent in May and June and continued sporadically through October (Figure 1). The external clinical signs of the disease were pink to white gills, exophthalmia, swollen abdomen, and mottled skin pigmentation. Affected fish had gray to tan livers and pink kidneys and spleens. The stomachs were often filled with a clear fluid and usually there were incidences of the intestine telescoping within itself (intussusceptions); the body cavity or intestines sometimes contained bloody fluid. The hematocrit of moribund fish ranged from 0 to 5%. Severely affected fish were commonly motionless at the pond edges. Weekly mortalities were as high as 5% in individual ponds. Some populations of affected fish were active and fed vigorously, while in others the fish would refuse feed even though they appeared to be hungry. Introduction of fresh feed into the latter populations would induce vigorous feeding activity. No bacterial, protozoal, or viral pathogens were

TABLE 2.—Occurrence of anemia in channel catfish fry fed test diets A_1–A_6 from farms with anemic fish. Diets C_1–C_3 were from fish farms that showed no evidence of the disease. Diets S_1 and S_2 were fry diets in use at the Southeastern Fish Cultural Laboratory.

	Number of days on feed									
	14		28		42		56		69	
Diet	N[a]	Anemic (%)	N	Anemic (%)	N	Anemic (%)	N	Anemic (%)	N	Anemic (%)
C_1	5	0	[b]							
C_2	5	0	69	0	91	0	70	0	585	0.34
C_3	5	0	65	0	99	0	70	0	683	0
A_1	5	0	59	3.4	80	0	70	1.4	645	0.16
A_2	5	0	65	0	96	0	70	2.9	557	0.36
A_3	5	0	67	4.5	84	1.2	70	1.4	627	0.96
A_4	5	0	65	3.1	92	2.2	681	0.4	[c]	
A_5	5	0	65	0	94	0	639	0	[c]	
A_6	5	0	45	11.1	103	0.97	70	4.3	596	3.19
S_1	5	0	65	0	100	0	70	0	651	0
S_2	5	0	67	0	88	0	70	0	618	0

[a] Number of fish checked.

[b] All fish lost for unknown causes; no evidence of anemia.

[c] Feed supply exhausted.

consistently associated with the disease. Infections of *Aeromonas hydrophila, Flexibacter columnaris,* or *Edwardsiella ictaluri* occurred in 26% of the populations affected by the anemic condition.

In efforts to determine the cause of anemia, feed was changed in 29 ponds, and as a consequence, mortality in 27 ponds ceased within 1 week. *Flexibacter columnaris* or gill parasites were associated with the anemia in the two ponds in which mortalities continued. Pond treatments with copper sulfate or potassium permanganate appeared to have no effect on mortalities unless accompanied by a change in feed, a reduction in feeding rate, or a cessation of feeding. The disease was observed in fish being fed every major brand of feed used in west-central Alabama. Feeds contained 26, 32, or 36% total protein and some contained 1 g oxytetracycline per 454 g feed. Selected feed samples from affected farms contained less than 0.33 μg/g lead, and no measurable amounts of insecticide, aflatoxin, or zearalenone. Diet A_6 which was associated with a high incidence of anemia contained 40.3 mg/kg pantothenic acid and 222 mg/kg vitamin E; within the range recommended for catfish. The physical and chemical characteristics of affected ponds varied greatly. No consistent pattern for water chemistry was evident in affected ponds.

Laboratory Experiments

No evidence of anemia was observed in subadults during the 97-day feeding experiment in the laboratory. Mean hematocrits on day 97 ranged from 36.5 ± 4.7% (SD) to 41.3 ± 3.0% (Table 1) in the experimental groups; values that compared favorably with the 35.9 ± 2.9% hematocrit for the fish before experimental feeding began. No individual hematocrits were near the extremely low range (0–5%) observed in the anemic fish and no mortality could be attributed to anemia. Fish fed diet A_6 rejected an estimated 25–30% of the daily ration which resulted in a lower growth rate (Table 1). Fish in all other tanks consumed the entire ration.

Anemic fish were observed in fry groups fed diets C_2, A_1, A_2, A_3, A_4, and A_6 (Table 2). On day 69, a total of 30 anemic fish from the experiment were isolated. Mean hematocrit of 15 of the anemic fish selected at random was 11.7 ± 5.2%. For comparison, hematocrit was 29.9 ± 4.8% for 48 fish selected at random from those fed diet S_1, and 31.7 ± 3.1% for 48 fish from those fed diet S_2. The remaining 15 anemic fish were isolated in a tank and placed on diet S_1. Hematocrit of the 13 fish that remained after 90 days on diet S_1, was 28.8 ± 3.7%, indicating that the fish had recovered. The "A" diets generally gave lower growth rates and higher mortalities than control diets (Table 3). All fish fed diet C_1 died 16 days after feeding began. A second group of fry immediately placed on diet C_1 also died during the third week of feeding. The cause of the mortalities was unknown. There was no evidence of anemia, parasitism, or bacterial infections.

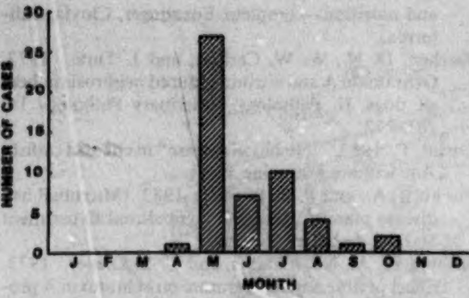

FIGURE 1.—Monthly occurrence of severe anemia cases in channel catfish reported by farmers in west-central Alabama during 1983.

TABLE 3.—Average weight, cumulative mortality, and daily weight gain of channel catfish fry fed experimental diets A_1, A_2, A_3, and A_6 and control diets C_2, C_3, S_1, and S_3 for 69 d.

Diet	*N*	Weight[a] (g)	Cumulative mortality (%)	Growth (g/d)
C_2	585	0.381	6	0.004
C_3	683	0.480	1	0.005
A_1	645	0.407	0	0.004
A_2	557	0.309	18	0.003
A_3	627	0.371	33	0.004
A_6	596	0.364	33	0.004
S_1	651	0.844	2	0.011
S_2	618	0.871	1	0.011

[a] Calculated from group weight and number.

Discussion

The cause of the anemic condition was not determined. The inducement of anemia in channel catfish fry fed diets from farms where the disease occurred, suggested that the causative agent was either contained within or associated with the feed and not with the pond environment. Since moldy pellets were observed in feed fed to the channel catfish fry in the laboratory, and in many feeds from affected farms, a mold toxin was considered a possible cause of the anemia.

Mold toxins such as ochratoxin A and B, citrinin, and aflatoxin have been found in animal feed and their effects on animals reported (Doster et al. 1972; Sansing et al. 1973; Smith et al. 1974; Kitchen et al. 1977; Ensminger and Olentine 1978; Visconti and Bottalico 1983). Possibly an unidentified mycotoxin attacks the hematopoietic tissues in channel catfish. The increase in hematocrit of anemic fish that were placed on a control diet in the laboratory experiments indicated that recovery from the disease by individual fish was possible when the feed was changed. This reinforces the field observation that pond mortality ceased when feed was changed. Several of the feeds associated with the anemia were reasonably fresh, showing no signs of mold or other spoilage. However, feed that appears fresh may contain pockets of contamination that are not detected in samples taken for analysis. There is also the possibility that other causes, such as a nutrient deficiency or a pathogenic agent, were involved.

Although diet A_6 induced anemia in catfish fry, it did not do so in subadults in the laboratory. The rejection of a portion of the daily ration of diet A_6 by the fish indicated that the feed was not palatable. The reduced feeding was reflected in lower growth rates for catfish on diet A_6 compared with diets C_3 and S_3 (Table 1). The fish may have been able to detect a contaminant in the feed that caused them to reject a portion of the daily ration, and they may not have received enough toxin to induce anemia. In addition, the number of adults (40) fed test diet A_6 may have been too small to detect anemia occurring at a low incidence.

Channel catfish fry could not be maintained on diet C_1 beyond 2.5 to 3 weeks of feeding. Clearly, an essential nutrient required by fry was lacking in this diet. Diets C_1–C_3 and A_1–A_6 were pelletized diets designed for subadult and adult catfish and were in use at farms in the affected area. Growth of catfish fry fed diets C_2, C_3, and A_1–A_6 was lower than that of catfish fry fed salmon and trout fry diets S_1 and S_2 (Table 3). Since there were no fry diets implicated in the anemia we ground the pelletized adult diets for feeding to fry. The protein content of the adult diets may not have been high enough to sustain good growth in fry. However, two of the adult diets (C_3 and A_5) did not induce anemia in catfish fry, suggesting that the adult diet formulation fed to fry did not itself induce the severe anemic condition.

Recommendation

Catfish farmers should strive to maintain freshly formulated feed under cool and dry storage. Bulk or bagged feed must be cooled to ambient temperature before storage. Old, moldy feeds should be removed and destroyed to prevent contamination of new feeds. Feed purchases should be planned to allow complete utilization in less than 60 d. Further research on anemia in catfish should include assays for mycotoxins or other contaminants and possibly for the presence of pathogens in feeds.

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A Two-Choice Water Recirculation Tank for Assessing Chemosensory Preferences of Landlocked Sea Lampreys

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Abstract.—A two-choice preference tank designed for assessing the responses of landlocked sea lampreys (*Petromyzon marinus*) to potential chemical attractants and repellents is described. The solutions in the experimental and control compartments of the tank are recirculated at the same rate permitting small quantities of substances to be tested at constant concentrations. No mixing between the two compartments was observed during 90-min dye tests. This tank should be suitable for preference tests with other aquatic organisms, similar in size to landlocked sea lampreys. Color-coded fluorescent tags, which permit identification of individual lampreys under ultraviolet illumination, and a simple timing system, which allows the time spent in the experimental compartment to be monitored for six lampreys, are also described.

A variety of techniques has been developed for assessing the behavioral responses of fishes to chemical stimuli, particularly with respect to avoidance of pollutants (Larrick et al. 1978). Although a number of these devices are also suitable for the study of potential attractants, including sex attractants (Timms and Kleerekoper 1972), most studies concerned with sex pheromone mediated behavior have relied upon observations of overt sexual behaviors such as courtship displays or spawning activity in small aquaria (Tavolga 1956; Stacey and Liley 1974; Partridge et al. 1976; Thiessen and Sturdivant 1977; Crow and Liley 1979). Two-choice mazes (Newcombe and Hartman 1973) and stream tanks (Emanuel and Dodson 1979) have been used to test potential sex attractants; however, these devices utilize flowing water and consequently require relatively large quantities of test substances.

The two-choice preference tank described below was designed primarily for use in experiments to isolate and identify sea lamprey (*Petromyzon marinus*) pheromones, the existence of which had been inferred from studies using flow-through preference tanks (Teeter 1980). Primary design consid-

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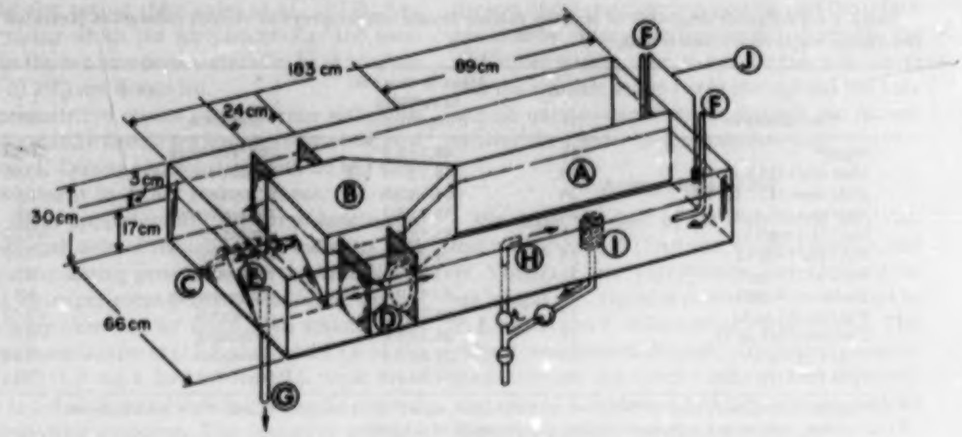

FIGURE 1.—Schematic diagram of a water recirculation tank for two-choice preference testing.

A tank divider
B pentagonal tank divider
C common end of tank
D funnel passage
E wedge-shaped barrier with drain holes
F inlet tubes for continuous flow into control compartment
G drain
H outflow of recirculation pump
I inflow of recirculation pump
J air supply to experimental and control compartments

erations in building the water recirculation tank were: 1) minimal mixing between solutions in the experimental and control compartments; 2) the ability to test constant, known concentrations of substances available only in limited quantities; and 3) the ability to test several lampreys simultaneously.

Tank Design

The preference-testing tank consists of a rectangular box constructed from 1.9-cm (¾-in) exterior grade plywood (Figure 1). The tank is divided into two compartments by a 30-cm (12-in) sheet of 6-mm-thick (¼-in), white Plexiglas (A), extending 89 cm (35 in) from one end. An irregular wedge-shaped pentagon (B), also constructed from white Plexiglas, reduces the width of each compartment from 33 cm (13 in) to 15 cm (6 in) at the common end of the tank (C) where the interface between the solutions in the two sides of the tank occurs. Two truncated, plastic funnels (D), connected at their narrow ends and sealed into the narrow part of each compartment with aquarium sealer, further reduce the connecting passage to a circular opening 5 cm (2 in) in diameter. The common chamber is divided at its center by a wedge-shaped Plexiglas barrier (E) which has 14 3-mm drain holes on each side, 1 cm (⅜ in) below the apex. The tank is sealed with two coats of epoxy paint (white provides the best contrast with darkly colored fish) and covered with a sheet of clear Plexiglas.

The water level in the tank is maintained at a depth of 20 cm (8 in), which is 3 cm (1¼ in) above the apex of the barrier wedge (E) in the common chamber (C). An inflow tube (F) in the upstream end of each compartment is used to provide a slight flow of water when that compartment is randomly selected to be the control compartment. This continuous flow of water is critical for maintaining separation of the solutions in the two compartments. The optimum flow to achieve this separation was empirically determined to be 200 mL/min (0.05 gal/min) for our test conditions. This flow is set with a precision flowmeter and excess water is removed at the same rate through the drain holes in the barrier in the common chamber (C). The drains empty into the space below the barrier (E) and the water then exits through a drain line at a rate controlled by a needle valve (G). The water in the stimulus and control compartments is kept in constant, vigorous motion by two matched pumps (Little Giant Pump Company, 4-MD) running at 50 L/min (13.2 gal/min). The output of each pump (H) is located about 39 cm (15½ in) from the funnels and the flow is directed toward the closed (upstream) end of the tank (Figure 1). The inflows of the pumps (I) are attached

TABLE 1.—Preference responses of sexually mature female sea lampreys to various substances presented in the two-choice water recirculation tank.

Test substance	N	Mean % time±SE in stimulus compartment	Response	P[a]
Water[b]	16	48.5±6.9	No preference	NS
Male urine (25.6 μL/L)[c]	24	68.7±7.1	Attraction	<0.01
Male urine (12.8 μL/L)[c]	24	64.5±6.8	Attraction	<0.05
Male urine (6.4 μL/L)[c]	24	55.8±8.3	No preference	NS
NaCl (1.8 mg/L)	10	85.9±4.7	Attraction	<0.02
NaCl (0.18 mg/L)	16	74.3±6.6	Attraction	<0.05
NaCl (0.018 mg/L)	15	53.2±7.5	No preference	NS
Na_2SO_4 (1.8 mg/L)	16	47.4±9.8	No preference	NS
Pyridine (67 μg/L)	12	33.3±8.5	Avoidance	<0.02
Dodecanol (83 μg/L)	12	26.2±4.9	Avoidance	<0.01
TFM (7.4 mg/L)[d]	10	29.6±3.3	Avoidance	<0.01
TFM (1.85 mg/L)[d]	14	51.8±8.9	No preference	NS

[a] Probablities determined using the Wilcoxon matched-pairs, signed-ranks test (two-tailed); NS = not significant.

[b] Control tests with no test substance in either compartment of the tank.

[c] Urine collected from sexually mature male sea lampreys. Samples contained no visible milt.

[d] TFM = 3-trifluoromethyl-4-nitrophenol.

to perforated plastic bottles, located in the bottom of the tank, 20 cm (8 in) from the closed end. This arrangement produces a swirling movement of water in each compartment, and the bottles prevent the lampreys from being sucked into the inlet ports. Each compartment is supplied with an airstone (J) and the temperatures of the solutions are continuously monitored with thermistors in the center of the experimental and control compartments.

Tags and Timing System

Lampreys are tested in groups of six of the same sex; each is marked with a color-coded tag. The color code is repeated with each group of lampreys. Tags are made by mixing non-toxic, fluorescent paints with silicone aquarium sealer and forming long strips of different colors by ejecting the colored sealer from a large plastic syringe. After curing, the strips are cut into 3–4-mm (about ⅛-in) lengths and threaded onto Floy tags in six distinct color combinations. When attached to the dorsal fin, the tags are readily visible in the test tank when the lampreys swim under a 40-W ultraviolet bulb suspended over the common end of the tank. Six elapsed-time indicators, operated by a series of toggle switches marked with the same color code as the lampreys, are used to record the time each lamprey spent in the experimental compartment during a test.

Operation

During extensive tests with a dye in the experimental compartment, virtually no mixing was observed between the solutions in the two sides of the tank during 90 min, the longest period tested. Disturbances of the interface between the solutions in the two sides of the tank, caused by swimming movements of the lampreys, were minimal and transitory. The slight inflow of water into the control side of the tank, which exited through the drain holes at the top of the barrier between the two compartments resulted in a net movement of water out of the control compartment. This helped maintain separation between the solutions in the two compartments of the tank.

Typically, six lampreys of the same sex, which have been held together for at least 24 h, are placed in the tank and given 10 min to habituate. During this period, the lampreys are permitted to swim freely throughout the tank. At the end of the habituation period, a stimulus is added to one of the two compartments, chosen at random, and an additional 5-min period is allowed to complete mixing of the test substance in the experimental compartment. In most cases, the lampreys actively explore both the experimental and control compartments during this time. If a lamprey does not enter both compartments during the first 3 min of the stimulus-equilibration period, it is gently forced into the other compartment with a net. The time each lamprey spends in the experimental compartment is then monitored during a 30-min test period with the elapsed-time indicators. The lampreys, which have a tendency to attach to the sides of the tank, are induced to swim by turning on a 100-W incandescent light suspended over the center of the tank for 30 s at 5-min intervals throughout the test period (McCauley et al. 1977). Any tests during which the temperatures of the solutions in the two compartments differ by more than 0.5°C (0.9°F) are discarded.

Representative results of preference tests with sexually mature female sea lampreys are presented in Table 1. During control tests, with no test substance present in either compartment, the lampreys spent approximately the same amount of time on each side of the tank. This indicates that neither the testing procedure nor the tank introduced an experimental bias. Significant preferences were observed for urine from sexually mature male sea lampreys (12.8 μL/L and 25.6 μL/L) and NaCl (1.8 mg/L and 0.18 mg/L), while male urine at 6.4 μL/L and Na_2SO_4 (1.8 mg/L) evoked no observable response. The lampreys avoided pyridine (67 μg/L), dodecanol (83 μg/L) and the larvacide, 3-trifluoromethyl-4-nitrophenol (TFM, 7.4 mg/L). TFM at 1.85 mg/L evoked no observable response.

Interactions between lampreys tested together appear to be limited to occasional physical encounters if two animals attempt to pass through the funnels at the same time. The lampreys show no apparent hesitation in swimming through the funnels or over the barrier in the common end of the tank. Although spawning-run male sea lampreys often avoid water in which other sexually mature males have been held, no obvious avoidance responses or agonistic behavior was observed in the preference tank between males held together for at least 24 h prior to testing (Teeter 1980). Spawning-run female lampreys show no obvious response to water in which other females have been held. The preference responses observed in the recirculation tanks are very similar to those obtained with approach–avoidance tubes and flow-through, two-choice tanks (Teeter 1980).

Utility

This preference-testing tank should be suitable for assessing the chemosensory preferences of other aquatic organisms similar in size to landlocked sea lampreys—30–45 cm (12–18 in) long and 10 cm (4 in) in girth. It is particularly useful for bioassays of natural products, or of fractions isolated during attempts to characterize pheromones, because materials available in small quantities can be tested at known constant concentrations.

The tank could be modified to accommodate either larger or smaller species by making appropriate adjustments in the overall size of the tank, the diameter of the openings through the funnels, the size of the recirculation pumps, and the inflow and outflow rates. In adapting this sytem for use with other species, care should be taken to insure that the animals move freely throughout the tank and do not hesitate to swim through the funnels or over the barrier in the common end.

Acknowledgments

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TECHNICAL NOTES

A Method for Testing Aerators for Fish Tanks

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Several types of aerators are used in tanks for holding and hauling fish, but few data are available for comparing the oxygen-transfer capabilities of these aerators. A procedure recommended by APHA et al. (1980) for determining oxygen-transfer rates of aerators for wastewater treatment was modified slightly and used to test four different aeration systems for fish-hauling tanks. The oxygen-transfer test outlined below can be used by fish culturists to compare the oxygen-transfer capabilities of other aeration systems.

Aerators should be tested in the hauling tanks. Fill the tank to normal capacity with a measured volume of water—preferably clear well water. Determine the dissolved oxygen (DO) concentration in the tank with a polarographic DO meter and measure the water temperature. Estimate the DO concentration at saturation; instructions presented by one of the following may be used: APHA et al. (1980), Soderberg (1982), or Colt (1984). Mount the aerator in the tank. The water in the tank is deoxygenated with 0.1 mg/L cobalt chloride ($CoCl_2 \cdot 6H_2O$) and 10.0–12.0 mg/L sodium sulfite (Na_2SO_3) for each milligram per liter of DO (1 mg/L = 1 g/m^3). The cobalt chloride and sodium sulfite should be dissolved in a pail of water from the tank and splashed over the water surface in the tank. The aerator will effectively mix the chemicals in the test tank. Position the DO meter probe at mid-depth, and at least 15 cm (6 in) from the walls of the tank. Measure DO concentrations at 1-min intervals until the DO concentration reaches 75 to 85% of saturation. Values for DO need not be recorded until the DO concentration begins to increase. Greater intervals of time between measurements are permissible if the DO concentration does not rise rapidly.

Data collected in an oxygen-transfer test are provided in Table 1. The DO deficits are obtained by subtracting DO concentrations in the tank from the DO concentration at saturation. Plot the DO deficits versus time on one-cycle, semi-logarithmic graph paper (Figure 1). The plot should approximate a straight line, the slope of which is the oxygen-transfer coefficient. Select points 1 and 2 on the graph in Figure 1 to correspond to DO concentrations of about 10% and 70% of saturation, respectively. Compute the oxygen-transfer coefficient with the formula

$$(K_La)_T = \frac{\log_e(DO_1) - \log_e(DO_2)}{(t_2 - t_1)/60};$$

$\log_e$ = natural logarithm;
$(K_La)_T$ = oxygen-transfer coefficient at temperature of test water, h^{-1};
DO_1 = DO deficit at point 1 on graph, mg/L;
DO_2 = DO deficit at point 2 on graph, mg/L;
t_1 = time at point 1 on graph, min;
t_2 = time at point 2 on graph, min.

Water temperature influences oxygen-transfer. Adjust oxygen-transfer coefficients to 20°C (68°F) with the equation

$$(K_La)_{20} = (K_La)_T \div 1.024^{T-20};$$

T = water temperature for test, °C;
$(K_La)_{20}$ = oxygen-transfer coefficient at 20°C, h^{-1}.

Table 1.—Results of an oxygen-transfer test conducted in a 0.70-m^3 tank of water at 22°C (71.6°F). The dissolved oxygen (DO) concentration at saturation was 8.7 mg/L.

Time[a] (min)	DO in tank (mg/L)	DO deficit[b] (mg/L)
0	0.15	
1	0.2	8.5
2	1.2	7.5
3	2.7	6.0
4	3.3	5.4
5	4.25	4.45
6	4.8	3.9
7	5.35	3.35
8	5.95	2.75
9	6.35	2.35
10	6.65	2.05
11	6.85	1.85

[a] Aerator started and DO concentrations measured at 1-min intervals. Timing began when DO concentration began to rise.

[b] DO concentration at saturation minus DO concentration in tank.

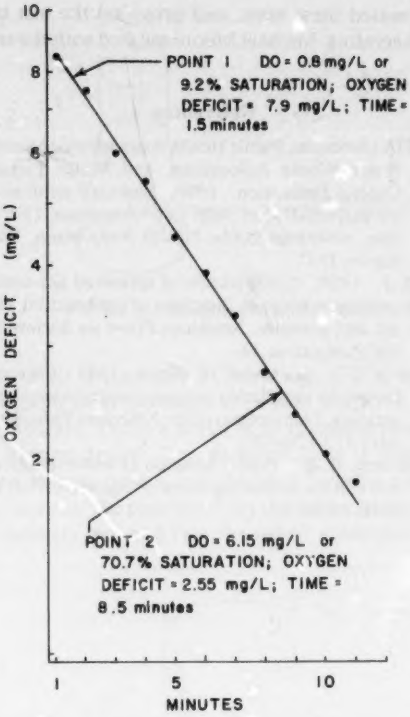

FIGURE 1.—Semi-logarithmic plot of dissolved-oxygen (DO) deficit values and aeration time (data from Table 1).

Calculate the oxygen-transfer rate for standard conditions (0 mg/L DO, 20°C, clear water) with the formula

$$OT_S = (K_La)_{20} \times DO_{20} \times V;$$

OT_S = standard oxygen-transfer rate, g O_2/h;
DO_{20} = DO concentration at saturation for 20°C and standard pressure, mg/L;
V = volume of water in tank, m^3.

If desired, the oxygen-transfer rate may be divided by power (horsepower or watts) to facilitate comparisons of different-sized aerators.

Data from Table 1 and Figure 1 are used in the following example for the calculation of an oxygen-transfer rate. First, compute the oxygen-transfer coefficient for the temperature of the test water (22°C)

$$\begin{aligned}(K_La)_T &= \frac{\log_e(7.9) - \log_e(2.55)}{(8.5 - 1.5)/60}\\ &= \frac{2.07 - 0.94}{0.117}\\ &= 9.66\ h^{-1}.\end{aligned}$$

TABLE 2.—Standard oxygen-transfer coefficients and rates in 20°C (68°F) tap water, 0 mg/L dissolved oxygen, for four aeration systems used in fish-hauling tanks. Each aerator was tested three times.

Aerator	Oxygen-transfer coefficient (h^{-1})[a]	Oxygen-transfer rates (g O_2/h)[a]
Micro-Por diffuser	7.01 z	55.3 z
Fresh-Flo	5.53 y	44.0 y
Crescent	5.26 y	41.3 y
McDonald	2.86 x	22.7 x

[a] Values indicated by different letters (vertical comparisons only) were declared significantly different ($P < 0.05$) by Duncan's multiple-range test.

Next, adjust the oxygen-transfer coefficient to 20°C

$$\begin{aligned}(K_La)_{20} &= 9.66 \div 1.024^{22-20}\\ &= 9.66 \div 1.024^2\\ &= 9.66 \div 1.05\\ &= 9.20\ h^{-1}.\end{aligned}$$

Finally, compute the standard oxygen-transfer rate. The DO concentration at 20°C and 100% saturation is 9.07 mg/L, the tank volume is 0.70 m^3, and

$$OT_S = (9.20)(9.07)(0.70)$$
$$OT_S = 58.4\ g\ O_2/h.$$

The oxygen-transfer rate from a standard test indicates the ability of an aerator to transfer oxygen to clear water at 20°C when the DO concentration is 0 mg/L. Oxygen-transfer rates are valuable for comparing aerators. However, in practice, aerators will not transfer oxygen at the standard rate, primarily because DO concentrations will be above 0 mg/L in tanks containing fish. The oxygen-transfer rate of any aerator decreases as the DO concentration rises, because the difference between the DO concentration at saturation and the actual DO concentration is the driving force causing oxygen to enter water. Shelton and Boyd (1983) presented procedures for adjusting standard oxygen-transfer rates for actual conditions.

The following aerators were tested: Fresh-Flo® Model TT, 12 V, 8.7 A (Fresh-Flo Corporation, Cascade, Wisconsin); Crescent® Model 7, 12 V, 3.5 A (Crescent Manufacturing Company, Fort Worth, Texas); McDonald Oxidator® Model 3, 12 V (McDonald Company, Erin, Tennessee); Micro-Por® diffuser (Micro-Por, Incorporated, Wichita, Kansas). The Micro-Por diffuser system consisted of a 57-cm-long × 1.5-cm-outside-diameter (22.5 in × ⅝ in) Micro-Por diffuser connected by rubber tubing to the pressure regulator of an oxygen cylinder. Oxygen was introduced at 0.35 kg/cm^2 (5 lb/in^2) and the diffuser was submerged to a depth of 81 cm (32 in). The paddle of the Crescent aerator was submerged to half its length during tests. The other two aerators could only be mounted and operated in one position. All tests were conducted in a fish-hauling tank with a capacity of 871 L (230 gal) and a depth of 81 cm (32 in). The tank was filled before each test with clear, aerated well water at 22°C (71.6°F). Each aerator was tested three times.

Standard oxygen-transfer rates are summarized in Table 2. The Micro-Por diffuser system transferred more oxygen than the others, and the McDonald oxidator transferred the least oxygen. The Fresh-Flo and the Crescent aerators did not differ in oxygen transfer rate. Other factors to consider are water circulation, cost, durability, and reliability. Probably the best aeration system for hauling fish would be a diffuser system to introduce oxygen into the water and a paddlewheel-type aerator (Fresh-Flo and Crescent are of this type) to circulate water and remove carbon dioxide.

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Paul Loska and Mickey Newman, Fisheries Division, Georgia Department of Natural Resources requested these tests, and provided the test tank and aerators. Michael Moore assisted with the tests.

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An Air-Powered Device for Dispensing Fluorescent Marking Pigment

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Fluorescent pigment marking of fish is a common tool for identifying groups of fish throughout their lives in a lake or stream. This technique uses high pressure air to imbed microscopic fluorescent granules in a fish's epidermis. The granules can then be seen for several years by examining the fish under an ultraviolet light.

Fish marked with these pigments may be periodically sampled to measure growth and survival rates. Creel census clerks may record numbers of marked fish caught by anglers to determine return to the creel, and marked fish may be stocked in a reservoir and the tailwaters sampled to determine escapement from the impoundment. Fish must be marked by consistently correct and efficient applications of the pigment. One of the major problems with the technique has been to prevent compaction of the pigment in the container—keeping it dry and fluffy so that the flow through the delivery gun is constant. If the pigment is contained in a hopper attached to the application gun, continuous agitation of the gun–hopper unit by the applicator is required. If the pigment is contained in a separate hopper, an additional crew member is required in order to stir the material throughout the marking procedure.

This article describes a device which uses air pressure produced by the marking gun compressor to stir and then dispense the pigment, eliminating the need for constant agitation of the pigment by a marking crew member, and ensuring a constant flow of pigment through the marking gun. The device is constructed of materials available in most hardware or automotive stores, and requires a basic knowledge of cutting, bending, and brazing for fabrication.

During fluorescent pigment marking, com-

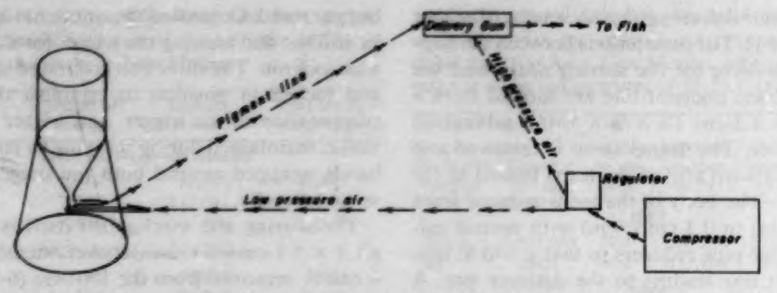
Delivery Gun
To Fish
Pigment line
High pressure air
Regulator
Low pressure air
Compressor

FIGURE 1.—Diagram of power source and of stirring and dispensing hook-ups for canister.

pressed air is provided at both high (7,756 mm Hg or 150 lb/in²) and low (776 mm Hg or 15 lb/in²) pressure. The low pressure air is used to power the stirring device. Dry, fluffy pigment is placed in the stirring device and the high pressure air is used to draw the pigment from the stirrer, through the delivery gun and into the fishes' epidermis.

The stirring and dispensing device is a cylindrical hopper supported on a stand. The hopper is made of a 30 cm (12 in) length of 20-cm (8-in) diameter heating duct brazed to a 20-cm (8-in) diameter metal funnel. Thin-walled 1.9-cm (¾-in) electrical conduit is bent and brazed to form the stand. The hopper is connected to both the com-

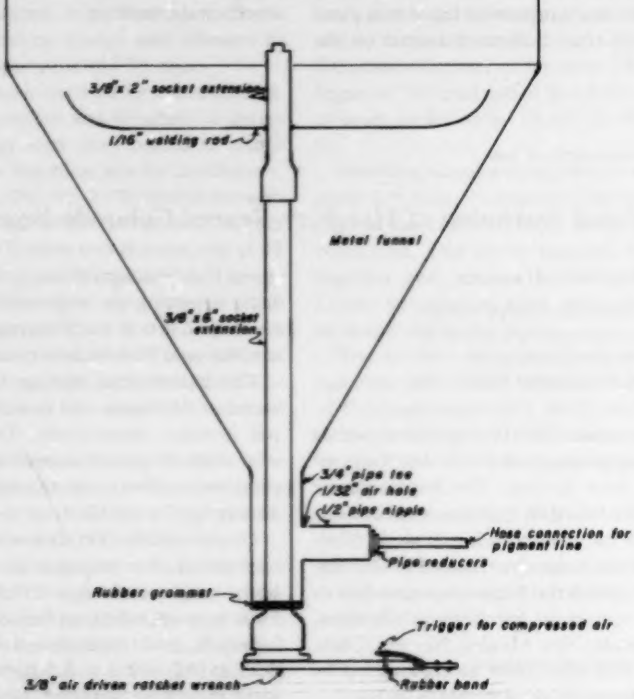
3/8" x 2" socket extension
1/16" welding rod
Metal funnel
3/8" x 6" socket extension
3/4" pipe tee
1/32" air hole
1/2" pipe nipple
Hose connection for pigment line
Pipe reducers
Rubber grommet
Trigger for compressed air
3/8" air driven ratchet wrench
Rubber band

FIGURE 2.—Cut-away overview of stirring and dispensing device and schematic of hook-up system. Measurements are given in inches (").

pressor and the delivery gun with lengths of rubber hose (Figure 1). The connections between the hopper and the drive for the stirring arms, and the hopper and the pigment line are formed from a 1.9 × 1.9 × 1.3-cm (¾ × ¾ × ½-in) galvanized water pipe tee. The funnel spout is removed and one 1.9-cm (¾-in) arm of the tee is brazed to the funnel base. The body of the tee is reduced from 1.3 cm (½ in) to 0.3 cm (⅛ in) with several galvanized water-pipe reducers so that it will fit into the pigment line leading to the delivery gun. A 0.08-cm (1/32-in) hole is drilled in the body of the tee to provide replacement air to facilitate the flow of pigment to the delivery gun. The stirring mechanism is a 1.0-cm (⅜-in) air-driven ratchet wrench powered by very low pressure (776 mm Hg or 15 lb/in^2) from the marking compressor, with a 1.0 × 15.2-cm (⅜ × 6-in) socket extension forming the drive shaft. The socket extension is fitted, near the socket head, with a rubber electrical grommet, and the shaft of the extension inserted through the other 1.9-cm (¾-in) arm of the tee and into the canister. The socket extension is then attached to the air-driven ratchet wrench (Figure 2).

The air-driven ratchet wrench is held in place under the hopper by clamps securing it to a piece of 3.9-cm (1½-in) iron channel mounted on the hopper stand. Control of the air ratchet is achieved by drilling and tapping the trigger for a stove bolt with lock nut. The stove bolt is screwed up or down and locked in position to establish the proper compression of the trigger, and trigger compression is maintained during spraying by large rubber bands wrapped around both the trigger and the wrench handle.

The stirring arm mechanism itself is based on a 1.9 × 5.1-cm (⅜ × 2-in) socket extension so that it can be removed from the 15.2-cm (6-in) socket extension which forms the drive shaft. The four stirring arms are constructed from 0.16-cm (1/16-in) welding rod, shaped to fit the funnel area of the hopper, and brazed to the shorter socket extension. Care should be taken to guarantee that the stirring arm mechanism is firmly attached to the drive shaft before the device is started.

The entire stirring and dispensing hopper is light weight and can easily be moved or lifted. The only maintenance necessary is periodic oiling of the air-driven ratchet wrench. The device has greatly improved the efficiency and uniformity of our spray-marking operations, and we recommend such equipment to anyone involved in fluorescent pigment spray marking.

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Induced Spawning of Hatchery-Reared Colorado Squawfish

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The Colorado squawfish (*Ptychocheilus lucius*) is presently being propagated at Dexter National Fish Hatchery, New Mexico. The largest native cyprinid in North America, this species is considered endemic to the Colorado River basin (Colorado River Fishes Recovery Team 1978). Deacon et al. (1979) listed the Colorado squawfish as an endangered species in the states of Arizona, California, Colorado, New Mexico, Nevada, Utah, and Wyoming. It is also listed as endangered by the U.S. Department of the Interior (1980).

The purpose of this study was to update the work conducted at Willow Beach (Arizona) National Fish Hatchery (Toney 1974; Hamman 1981). Data regarding the successful spawning of hatchery-reared brood stock during 1983–1984 at Dexter National Fish Hatchery are presented.

The brood stock during 1983 and 1984 consisted of 24, 9-year-old females and nine 10-year-old females, respectively. These fish were 1974 year-class progeny from wild adults collected from the Yampa River and spawned at Willow Beach Hatchery (Toney 1974).

Ovulation was induced with injections of acetone-dried carp pituitary at the rate of 4 mg/kg body weight at 24-h intervals (Hamman 1981). Fish were anesthetized before the intraperitoneal injection, and then placed in a 2.75 × 0.75 × 0.75-m (9.0 × 2.5 × 2.5-ft) holding tank and held until they were stripped. Cool water at 17–18°C (62–64°F) flowed through the tank at 90–130 L/min (23–34 gal/min). Natural spawning of Col-

TABLE 1.—Summary of spawning data for Colorado squawfish during May–Jun 1983 and 1984 at Dexter National Fish Hatchery, New Mexico.

Value	Weight of fish (g)	Eggs per female	Eggs per kg body weight
1983: 24, 9-year-old females			
Minimum	1,045	57,766	37,695
Maximum	2,045	113,341	66,452
Mean	1,403	77,400	55,533
1984: nine 10-year-old females			
Minimum	1,182	11,977	7,984
Maximum	1,727	91,040	61,135
Mean	1,464	66,185	45,451

TABLE 2.—Summary of data for hatching eggs of Colorado squawfish during 1983 and 1984 at Dexter National Fish Hatchery, New Mexico. All eggs were incubated at water temperatures of 20–22°C (68–71°F).

Incubation method	Number of eggs	% viability at 48 h	Expected number of fry	Actual number of fry	% of expected
1983					
Heath	1,625,029	59.1	960,392	283,017	29.4
Jar	232,577	66.2	153,965	34,855	22.6
1984					
Jar	595,672	48.9	291,283	118,157	40.5
Totals	2,453,278	57.3	1,405,650	436,029	31.0

orado squawfish is believed to occur after the water temperature reaches 21°C (70°F) (Vanicek and Kramer 1969).

When eggs could be expelled with slight pressure, a female was anesthetized and rinsed, and the eggs were stripped into a pan containing water and diluent. Milt from two or three hatchery-reared males was added, and the eggs were gently stirred with a feather. A bentonite clay solution was then added to counteract egg adhesiveness. Eggs were then washed and placed in a 31 × 31 × 18-cm (12 × 12 × 7-in) floating basket and allowed to water harden for approximately 30 min.

Using the gravimetric method for enumeration, eggs were sample counted and weighed to determine fecundity. Eggs were then placed in either Heath incubators or hatching jars for incubation. Heated water at 20–22°C (68–71°F) flowed through the jars and incubators at the rate of 4–11 L/min (1–3 gal/min). Egg viability was determined at 48 h to estimate hatching percentage.

After the fry hatched, they were transferred to 2.75 × 0.75 × 0.75-m (9.0 × 2.5 × 2.5-ft) tanks and held until swim-up occurred. Warm water at 20–22°C (68–71°F) flowed through the tanks at 15–23 L/min (4–6 gal/min). Fry were then counted and stocked.

In 1983, all 24 females ovulated 18–20 h after receiving one carp pituitary injection. Fecundity averaged 77,400; average number of eggs per kilogram body weight was 55,533 (Table 1).

Altogether, 1,625,029 eggs were placed in Heath incubators and 232,577 eggs were placed in incubation jars. Eggs commenced hatching at 100 h and were completely hatched by 144 h. Expected numbers of fry were estimated at 960,392 (Heath) and 153,956 (jars) or a 59.1% and 66.2% viability, respectively, at 48 h (Table 2).

After fry hatched, they were transferred to holding tanks. Fry were usually swimming to the surface 5–7 d after hatching. Actual numbers of fry were 283,017 (Heath) and 34,855 (jars) for 29.4% and 22.6% of expected fry, respectively.

In 1984, all nine females ovulated 20–24 h after receiving two hormonal injections. Fecundity averaged 66,185; average number of eggs per kilogram body weight was 45,451 (Table 1). All eggs were incubated in hatching jars (Table 2). Egg viability ranged from 40.7 to 55.8% (mean 48.9%). Expected numbers of fry were 291,283. Hatching began at 96 h and ended by 144 h. Actual numbers of swim-up fry were 118,157, or 40.5% of expected fry.

Hatching success compared to egg viability was good; however, fry mortality attributed to "bunching" in the corners of the holding tanks was encountered. The newly hatched fry would bunch together and, after a period of time, suffocate. Losses occurred at night when the fry could not be dispersed in the tanks.

The method of using Heath incubators or incubation jars should be suitable for hatching eggs of Colorado squawfish. Both methods of incubation can be applied, but a larger quantity of eggs can be hatched in less space, with minimal water, using jars.

The future of the Colorado squawfish may depend upon hatchery efforts to help restore the species. If culture techniques are developed now, this species may again inhabit its historic habitats in the Colorado River basin.

Acknowledgments

I thank Buddy Jensen, Troy Winham, Sharon Coats, Connie Foster, and Joe Owens for assistance during this study.

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The Progressive Fish-Culturist 48:74–75, 1986

The Penny Fry Trap

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Traps used to capture larval, fry, and juvenile fish have been developed by several workers (Casselman and Harvey 1973; Beard and Priegal 1975; Graser 1978; Trippel 1981).

The trap described here was developed to meet our need for an inexpensive portable device for sampling fry and juvenile populations of fish from the genera *Cyprinodon, Empetrichthys,* and *Crenichthys,* among others, in shallow nursery waters surrounding desert springs and their outflows. These traps have also been used successfully for related work on Caribbean islands.

The desert aquatic habitats are often less than 20 cm deep with a heavy growth of filamentous algae over a loose substrate of mud and silt. Conventional 6-mm-wire-mesh minnow funnel traps are too large for sites such as these, and the mesh allows the fry and juvenile fish of smaller species to escape.

The trap is inexpensive, portable, and easy to construct (Figure 1). Materials include fiberglass window screening, adjustable plastic embroidery hoops, and a silicone adhesive. Construction costs are about $2.00 per trap. Although actual construction time is only about 15 min per trap, several hours are required to allow the adhesive to dry.

Trap diameters of 10.5 and 15 cm were selected from several available hoop sizes. The trap lengths used were 20 and 25 cm.

To construct the larger trap, cut a 25 × 51 cm piece of screen, or 20 × 34 cm for the smaller trap. Spread a 5-mm-wide bead of adhesive thinly on the shorter edge of the screen. Form a cylinder by joining the two edges with a 1.5-cm overlap. Set the cylinder aside and allow the adhesive to dry for at least 2 h.

Cut two 27-cm-diameter discs from the window screen. Make a cut to the center of each disc. Apply adhesive to at least one edge of the cut and form each disc into a cone with a diameter of about 20 cm (15 cm for the smaller trap). Set the cones aside to dry for about 2 h. Cut off the tip of each cone about 1.5 to 2 cm from the apex to create a 2-cm orifice. Smaller or larger cuts can be used depending on the size orifice desired.

Cement the cones to the smaller, inner ring of the embroidery hoop by placing adhesive around the outer surface of the inner ring and slipping the inner ring over the cone until it is level and fits snugly. The large diameter end of the cone may be too large; trim excess material from this end until about 1 cm of material remains. It can then be folded up over the ring and held in place with a stout rubberband until the adhesive dries. The trap is assembled by slipping the cone rings into the screenwire cylinder and securing them in place with the adjustable outer rings.

The larger of the two traps is more flexible than desired but a strip of rigid plastic can be placed

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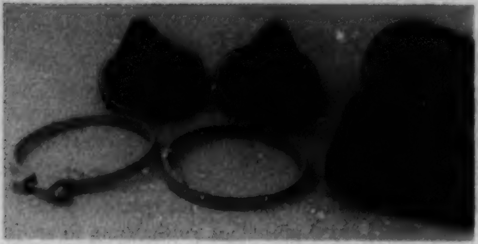

FIGURE 1.—Components of the Penny fry trap.

between the two outer rings to provide support. Strip dimensions are 28 cm long by 1 cm wide with a 5-mm hole drilled 1 cm from each end. The plastic strip is secured by the shafts on the adjustable outer rings. A third hole can be drilled in the center of the strip to secure a line for handling the trap. These traps are very light; placing a small rock or other weight inside the traps will help submerge them and hold them in place.

Fish are removed by inverting one of the cones or, if necessary, by removing one of the outer rings and cone. These traps have been effective in capturing fish from 8 mm to 6 cm long. If used in salt or brackish water, they should be washed in fresh water and all metal parts coated with a silicone lubricant.

Acknowledgment

We would like to thank Penny Baugh for suggesting the use of embroidery hoops.

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ERRATA

Please make the following changes in the paper "Culture of Hybrid Striped Bass to Marketable Size in Circular Tanks," by L. C. Woods III, J. H. Kerby, and M. T. Huish, that appeared in *The Progressive Fish-Culturist* 47:147–153, July 1985.

Abstract, page 147, line 4: change 3.34 kg/m^3 (260 lb/ft^3)
to 3.34 kg/m^3 (0.21 lb/ft^3).

line 6: change 3.02 kg/m^3 (235 lb/ft^3)
to 3.02 kg/m^3 (0.19 lb/ft^3).

Text, page 149, left column: make the same changes in lines 17 and 15, respectively.

The Progressive Fish-Culturist 48:77–79, 1986

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Prefixes

kilo (10^3)	k
milli (10^{-3})	m
micro (10^{-6})	μ

Time and Temperature

day	d
degrees Celsius ([°F − 32]/1.8)	°C
degrees Fahrenheit ([1.8 × °C] + 32)	°F
hour (spell out for diel time)	h
minute	min
second	s

Spell out year, month, and week.

Weights and Measures (Metric)

centimeter (0.394 in)	cm
gram (0.0353 oz)	g
kilogram (2.20 lb)	kg
kilometer (0.622 mi)	km
liter (0.264 gal, 1.06 qt) (exception to SI)	L
meter (1.09 yd, 3.28 ft, 39.4 in)	m

Weights and Measures (English)

cubic feet per second (0.0283 m^3/s)	ft^3/s
foot (30.5 cm)	ft
gallon (3.79 L)	gal
inch (2.54 cm)	in
mile (1.61 km)	mi
ounce (28.4 g)	oz
pound (0.454 kg, 454 g)	lb
quart (0.946 L)	qt
yard (0.914 m, 91.4 cm)	yd

Spell out acre (0.405 hectare), hectare (2.47 acres), ton (2,000 lb, 907 kg), and tonne (1,000 kg, 2,200 lb).

Mathematics and Statistics

all standard mathematical signs, symbols, and abbreviations	
base of natural logarithm	*e*
common test statistics (*F*, *t*, etc.)	
correlation coefficient (multiple)	*R*
correlation coefficient (simple)	*r*
degree (angular or temperature)	°
degrees of freedom	df
logarithm (specify base)	log
minute (angular)	′
not significant	NS
percent	%
probability	*P*
sample size	*N*
second (angular)	″
standard deviation	SD
standard error	SE

Physics and Chemistry

all atomic symbols	
alternating current	AC
ampere	A
British thermal unit (1.05 J)	Btu
chemical acronyms listed in Webster's dictionaries (DDT, EDTA, etc.)	
direct current	DC
footcandle (0.0929 lx)	fc
hertz	Hz
hydrogen ion activity (negative log of)	pH
joule (0.239 gram-calories, 0.000948 Btu)	J
lux (10.8 fc)	lx
molar	M
mole	mol
ohm	Ω
ortho	*o*
para	*p*
pascal	Pa
parts per million (per 10^6) (in the metric system, use mg/L, mg/kg, etc.)	ppm
parts per thousand (per 10^3)	ppt, ‰
siemens (=mho, Ω^{-1})	S
volt	V
watt	W

Calories should be converted to joules in the metric system.

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north	N
south	S
west	W
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et alii	et al.
et cetera	etc.
for example	e.g.,
months (tables, figures): first three letters (Feb, Jun, etc.)	
sex (tables, figures, hybrid crosses): female	♀
male	♂
that is	i.e.,
Union of Soviet Socialist Republics	USSR
United States (adjective)	U.S.
United States of America (noun)	USA

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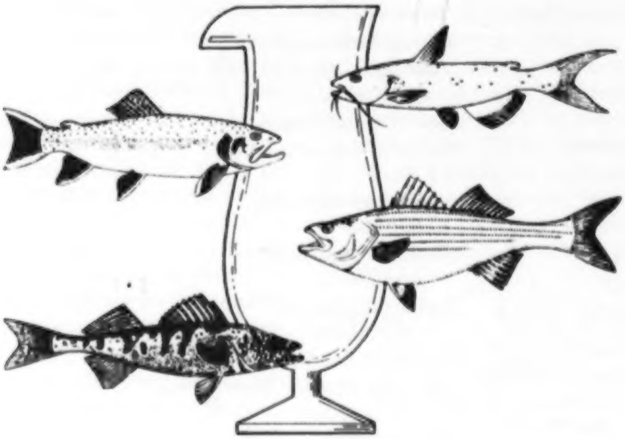

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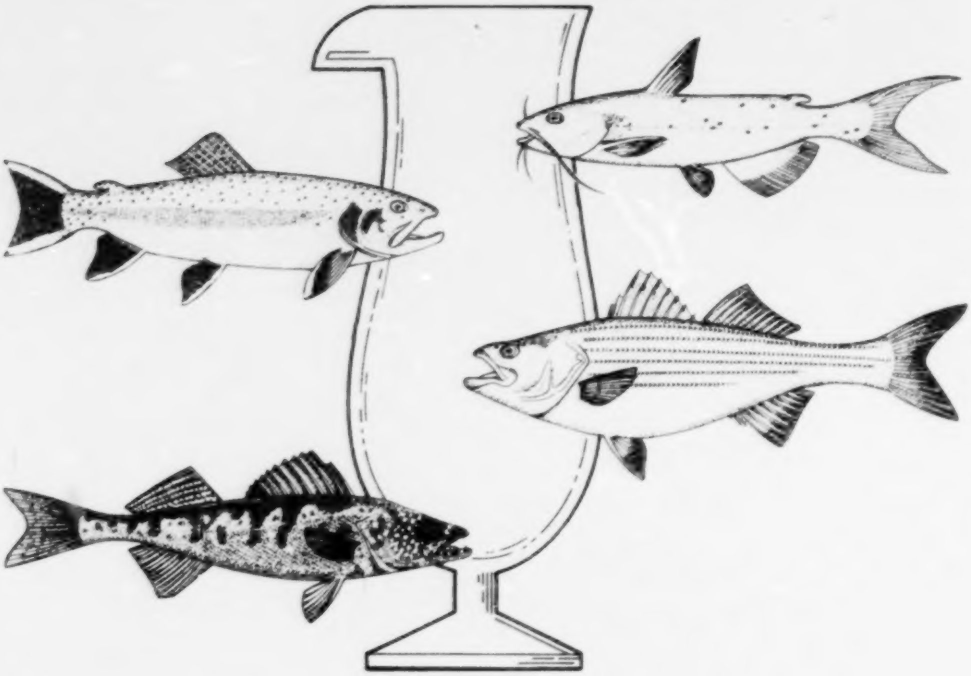

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Progressive Fish-Culturist

Volume 48 April 1986 Number 2

The Progressive Fish-Culturist 48:81–89, 1986

Review of the Intensive Culture of Walleye Fry

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Abstract.—Efficient intensive culture of larval walleyes (*Stizostedion vitreum*) may soon be realized due to results of research on larval development, culture environment, preferred food organisms, and feeding techniques. Larval behavior and morphology (developmental state) can be used as indicators for appropriate handling and feeding techniques. Certain environmental conditions such as a rearing unit color that contrasts with food organisms, water temperatures of 18–22°C, uniform lighting, and a stocking density of no more than 35,000 fish/m^3 reportedly contribute to increased growth and survival. Successful culture requires that suitable food such as cladocerans, copepods, and brine shrimp be continuously available at a high density (0.1 organism/mL). Cannibalism among walleye larvae has not been prevented but has been reduced through the use of suitable fish and food densities.

Culture procedures must augment survival and reduce intraspecific competition and cannibalism. Intensive culture of larval walleyes (*Stizostedion vitreum*) involves the manipulation of an environment designed to provide adequate water flow for the provision of oxygen and removal of metabolites, temperatures suitable for growth, and the regulated addition of suitable food. Beyerle (1975) reported that all attempts at intensive culture had been unsuccessful. However, research conducted over the last decade may help to make intensive culture of walleyes feasible.

In this review, we summarize current intensive culture techniques for larval walleyes. We discuss implications of developmental changes, environmental conditions, and various foods and feeding methods; identify problems related to high mortality; and offer some recommendations. The terminology used for larval stages is generally that described by Balon (1975)—an *embryo* is a fish that has not yet resorbed its yolk sac, and a *larva* is a fish that has begun feeding but has not yet begun metamorphosis to the juvenile stage. Temperature units (TU) are average daily temperatures (degree days) above 0°C, and TU totals are sums of daily TUs. Because a group of walleye eggs may hatch over a period of 2 or more days, the starting point of TU computation is taken to be the midpoint of the hatching period.

Larval Development

Some difficulties and uncertainties of intensive culture can be overcome by analysis and close observation of the morphological development of walleyes. Effective culture techniques depend upon how the fish are handled and fed during several critical periods during the delicate early life stages. It is important to monitor larval development in order to eliminate ill-timed handling and late or inappropriate food introduction.

Several stages of development are easily identifiable and are of particular interest to culturists.

TABLE 1.—Developmental conditions through first feeding, visual signs, and suggested culture techniques. TU = degree-days above 0°C.

Stage I: surface suspension (7–29 TU)

Visual signs
- Elongated yolk sac
- Surface suspension
- Swimming to the surface and drifting back to the bottom

Culture techniques: do not crowd fish for transport; avoid splash or turbulence on water surface; provide oxygen-rich water

Stage II: initiation of gill respiration (70 TU)

Visual signs
- Fish swim horizontally
- End of surface suspension
- Gills visible

Culture techniques: clean tanks if necessary; begin feeding (100 TU)

Stage III: initiation of feeding (120+ TU)

Visual signs
- Yolk absorbed
- Food in stomach

Stress syndrome
- Swimming in circular fashion, constantly in motion
- Lack of food in stomach
- Melanophores concentrated dorsal to gastrointestinal tract
- Fish against the walls, oriented away from tank center

Culture techniques: cause of swimming behavior is unknown and most affected fish will die; environmental factors are suspect

Stage IV: initiation of cannibalism (100–214 TU)

Visual signs
- Cannibalistic attacks
- Swim bladder inflated in some but not all fry
- Starving fish revert to prolarval behavior—swimming from the tank bottom to the surface

Culture techniques: feed natural zooplankton or large brine shrimp 1–3 mm long in addition to brine shrimp nauplii; reduce fish density

Each change in morphological stage is accompanied by changes in physiology and behavior (Table 1).

Stage I: Surface Suspension

The first stage (7–29 TU) begins shortly after hatching and during yolk absorption. The heart rate is reduced and blood flow patterns are altered. The result of the circulatory changes is maximum superficial exposure of blood in the subvitelline vein, at the ventral abdominal surface, where respiration occurs (McElman and Balon 1979; Balon 1981). Embryos characteristically suspend themselves at the water surface, vertically, with head up and the ventral abdominal surface of the yolk and oil near the surface. In nature, fish thus supported are transported in currents of streams or lakes (Eschmeyer 1950; Priegel 1970). If either water current or surface agitation is too high, embryos sink (Priegel 1970) but, with the aid of the buoyant oil globule, they are able to swim to the surface and resuspend themselves. Even though no apparent current or agitation is involved, some fish may sink motionlessly to the bottom and either struggle back to the surface or remain motionless on the bottom.

The reduced heart rate and slower blood flow may be beneficial, since blood will be exposed to oxygen-rich water for a longer time (McElman and Balon 1979). Energy conservation during surface suspension may contribute to increased survival later. During this first developmental stage, fish that are crowded (as for transport in bags or for volumetric displacement measurements) may incur delayed mortality (Colesante and Schiavone 1980). Throughout this stage, fish appear to be easily stressed or damaged and should be handled very carefully, if at all.

Stage II: Initiation of Gill Respiration

At about 70 TU, the second stage begins as the gills form and receive blood (McElman and Balon 1979). The fish begin to swim horizontally. Concurrent with the respiratory transformation, walleyes undergo other changes, which include lengthening and thickening of the lower jaw (up to 90 TU) and calcification of teeth at 81–94 TU (Olson 1974; McElman and Balon 1979). Completion of yolk-sac absorption occurs between 100 and 127 TU (Reighard 1890; Olson 1974; McElman and Balon 1979), and the fish culturist should offer food before yolk absorption is completed.

Stage III: Initiation of Feeding

The onset of exogenous feeding marks the start of the third stage. Olson (1974) stated that walleyes are ready to feed when the teeth calcify (81 TU) but most observers have placed the start of fish feeding at 100–122 TU (Reighard 1890; Olson 1974; McElman and Balon 1979). At first feeding, larvae are nearly transparent, so ingestion is easily observed.

If fish do not feed, they often exhibit a stress syndrome behavior (Beyerle 1979): the fish swim continuously in a circular motion as if they were swarming or schooling (Table 1). They often swim through water containing food organisms without feeding. Some fish may also move to the sides of the tank and face the tank wall. As seen from above, fish under stress are easily recognized by concentrations of melanophores dorsal to the gut and by an empty gastrointestinal tract. Larvae that starve for a few days after yolk absorption begin to digest the oil globule. McElman and Balon (1979) stated that absorption of oil appears to be a preservation strategy until exogenous feeding begins.

Stage IV: Initiation of Cannibalism

Cannibalism begins during the fourth developmental stage. Duration of this stage is not clearcut because cannibalism may begin concurrently with the initiation of exogenous feeding (120 TU) (Howey et al. 1980; Raisanen 1982) or as late as 214 TU (McElman and Balon 1979). The occurrence of cannibalism is reported from ages 6 to 16 d by Li and Mathias (1982), and ages 12 through 20 d by Cuff (1977, 1980). Populations of walleye fry can be decimated unless steps are taken to control cannibalism. Li and Mathias (1982) found that early cannibalism was related to fish densities greater than 15,000/m^3 and could be delayed beyond 200 TU if fish were reared at lower densities. At high densities all fish may not have an opportunity to feed, and density dependent stress may exist. Cuff (1977), using brine shrimp nauplii *Artemia* sp. as food, noted that increasing spatial separation among feeding and nonfeeding walleyes may reduce cannibalism even when food is abundant.

McElman and Balon (1979) associated cannibalism with swim-bladder inflation (214 TU) and an accompanying change in behavior. Once feeding walleyes inflate the swim bladder, they become more aggressive and tend to cannibalize either feeding fish that have not inflated their swim bladders or starving fish that have reverted to swimming high in the water column but periodically falling, and lying motionless on the tank bottom. Fish with uninflated swim bladders (up to 20% of the fish: Howey et al. 1980) normally orient toward the sides of the tank, whereas starving fish lie motionless in the water. Both conditions leave fish vulnerable to predation.

Rearing Environment

Rearing Unit Design

Beyerle (1975) stated that every conceivable container has been used in attempts to rear walleye. To date, no single appropriate rearing unit has been identified. A culture container with a water-upflow design may help keep live food organisms suspended in the water column. Nickum (1978) made this suggestion; however, no comparative data have been published. Colesante (1983) reared larval walleyes in an upwelling culture tank—a design used for culturing striped bass larvae *Morone saxatilis* (Lewis and Heidinger 1981). Colesante reported 20.4% survival for walleyes reared for 30 d in the upwelling unit and fed brine shrimp.

Color and Light Intensity

Colors within a culture system can have a striking effect on visual perceptions of larvae. Visual sensitivity in relation to color contrast of food and tank may be significant. Barker and Bryant (1981) showed that when common carp *Cyprinus carpio* were presented various food and background colors, the fish preferred green food particles and selected against red and blue particles. Common carp select feed colors that match the surrounding background. In contrast, Corazza and Nickum (1981) suggested that a green background might appear bright to walleye, thereby eliminating color contrast between the food and the background. Tank color or food color may influence the walleye's ability to feed efficiently. Corazza and Nickum (1981) compared walleye dispersal in tanks colored white, gray, green, or yellow. Walleyes were positively attracted to the sides of white tanks, but the best fish dispersal was in gray tanks; dispersal differed significantly from that in tanks of other colors.

Levine and MacNichol (1982) described the multipigment visual system in walleyes. Walleye vision is red and green sensitive, and by superimposing colors within the walleye's visual spectrum, contrast between prey and rearing tank can be enhanced or reduced. Normally, red and green objects would appear bright, while a dark gray object against a blue background becomes nearly invisible in green light.

Larval walleyes may not have the same visual orientation as the adult fish described by Levine and MacNichol (1982). Bulkowski and Meade (1983) pinpoint changes in phototaxis of larvae. Fry are photopositive until they reach a length of 32 mm. As walleyes grow beyond 32 mm, their behavior gradually changes and they seek low-light levels. The early phototrophic reaction is sufficiently strong that, once the fry are respiring through the gills, lights can be used to bring them up from the bottom, and tanks can be cleaned without harming the fish.

Woynarovich (1960) stated that, under normal room-lighting conditions, light levels of 400 lx or greater can be detrimental to the survival of embryos (4–6 mm long) of European pike-perch

TABLE 2.—Larval walleye development at different temperatures.

Observable characteristics of walleye development	Fish length (mm)	Temperature units[a]	Water temperature (°C)	Reference
Vertical swimming pattern (fish swim to surface, then fall to bottom, or remain suspended at water surface)	7–8.0	7.5–29	15	McElman and Balon (1979)
Peristalsis in gut	[b]	20	9.4	Reighard (1890)
		41	15	McElman and Balon (1979)
Respiration via gills	8.7–9.6	69	15	McElman and Balon (1979)
Termination of surface suspension		178.6	9.4	Reighard (1890)
Calcification of teeth	9.0	81	14–20	Olson (1974)
		93.8	15	McElman and Balon (1979)
Completion of yolk-sac absorption	[b]	100	15	McElman and Balon (1979)
		122.2	9.4	Reighard (1890)
		127	14–23	Olson (1974)
Recorded first feeding	9.0–9.7	101	15	McElman and Balon (1979)
		120–160	14–23	Olson (1974)
		122.2	9.4	Reighard (1890)
Inflated swim bladder	[b]	214	15	McElman and Balon (1979)

[a] Degree-days above 0°C.

[b] No length given.

(*Stizostedion lucioperca*), a species closely related to the walleye. High light levels cause day blindness and result in starvation unless the light is reduced when the fish reach the feeding stage. Normal room lighting, or bright lighting, may cause the fish to become disoriented, suspend themselves at the water surface, and eventually starve (Woynarovich 1960).

Tank covers also influence lighting within culture tanks. Howey et al. (1980) crowded walleyes into one-third of a rectangular tank by covering the remainder of the tank. Larvae, manipulated in this manner, can be crowded for presentation of food. However, Raisanen (1982) evaluated larval walleye survival in both covered and uncovered tanks; when tanks were covered, survival and food ingestion declined. Crowding and lighting were not assessed separately for effects on mortality and feeding. Raisanen's (1982) findings supported speculations by Nickum (1978) that uniform tank lighting is most beneficial for feeding larvae.

Water Flow

Turbulence should be sufficiently low to allow the surface suspension of embryos, and water flow should remain below the maximum swimming speed of the larvae. Houde (1969) measured swimming speed of larval walleyes and found that maximum swimming ability increased exponentially as the fish grew from 7 to 9.5 mm long. Maximum swimming speed for larvae 8.0–8.99 mm long averaged 1.3 cm/s and increased to 3–4 cm/s for 9.55-mm-long larvae. Flow velocities greater than the swimming speed would force larvae against outlet screens. Upwelling water flow, as discussed earlier, may offer a survival advantage.

Temperature

Survival of larval year classes in the wild depends upon the warming of water in the spring (Priegel 1970; Busch et al. 1975; Serns 1982) and, according to Smith and Koenst (1975), the same is true for hatchery-reared walleyes. In the Smith and Koenst studies, highest survival occurred among walleyes incubated at 9–15°C, then reared at 21°C. No walleyes survived at temperatures less than 18°C. Optimum growth temperature for juveniles is 22°C (Smith and Koenst 1975). Similarly, Hokanson (1977) stated that optimum yields of swim-up larvae occur when incubation temperatures were increased 1°C/d, thereby reducing hatching time and embryonic anomalies. Larval development at different temperatures is presented in Table 2.

Rearing Density

Stocking rates are usually expressed as density or number of fish per unit of volume. Olson (1974) reported that walleye density does not influence acceptance of zooplankton by the fish. Density does, however, affect the incidence of cannibalism. Larval rearing densities have been reported to as

TABLE 3.—Size of food particles ingested by walleyes 9–19 mm long.

Age of fish (d)	Approximate fish length[a] (mm)	Food particle size[a] (mm)			Type of food	Reference
		Minimum	Mean	Maximum		
5–6	9.0		0.30		Liquifry #1 diet	McElman and Balon (1979)
		0.33		0.39	*Bosmina*	Merna (1977)
		0.40		0.42	Brine shrimp	Howey et al. (1980)
				0.60	*Daphnia* spp.	Merna (1977)
				1.11	*Daphnia* spp.	Howey et al. (1980)
			0.25		W-7 dry diet	Nickum (1978)
			0.20		Brine shrimp cyst	Wheeler et al. (1979)
		0.90		1.10	*Cyclops*	Mathias and Li (1982)
				1.55	*Cyclops*	Raisanen and Applegate (1983)
7–8	9.3–9.4			0.9	Cladocerans Copepods	Merna (1977)
		0.45		0.75	*Moina*	Raisanen and Applegate (1983)
5–13	9.0–9.9			0.86	Cladocerans Copepods	Spykerman (1974)
13+	10.0	0.5			Copepods Cladocerans	Merna (1977)
		0.95		1.20	*Cyclops*	Mathias and Li (1982)
15	10.8–11.2	0.70		0.96	*Moina*	Raisanen and Applegate (1983)
		0.96		1.22	*Cyclops*	Raisanen and Applegate (1983)
16–25	11.2–18.8	0.98		1.24	Cladocerans	Mathias and Li (1982)
		0.98		1.95	*Cyclops*	Mathias and Li (1982)
				1.38	*Cyclops*	Raisanen and Applegate (1983)

[a] Measured along the longest axis.

high as 1,000,000/m^3 (Nickum 1978). At the National Fishery Research and Development Laboratory, we have observed that cannibalism and mortality are reduced at densities of roughly 4,000 to 5,000/m^3 in aerated units in which fish are not crowded under a light (unpublished). At low initial densities, less handling is required to reduce the number of fish as they grow. Howey et al. (1980) encountered problems with cannibalism when the larvae, at a density of 28,500/m^3, were crowded into one-third of the tank volume, which effectively tripled their density.

Li and Mathias (1982) noted that survival in 30-L aquaria decreased from 16%, when fry were stocked at 1,000/m^3, to 2%, when stocked at 10,000/m^3, to 0.3% when fry were stocked at 100,000/m^3. Raisanen and Applegate (1983) tested larval density in 115-L aquaria. Optimum density from these studies was 35,000 fish/m^3. At densities of 50,000–165,000/m^3, Colesante and Youmans (1981) encountered high rates of cannibalism and often total mortality, with cannibalism generally increasing with fish density. Walleyes apparently tolerate higher densities in rearing tanks larger than 30 L. Stress behavior, described by Beyerle (1979), was reduced as densities were reduced. At high fish densities, it is more difficult to provide a uniformly distributed, ample supply of food, even with live food organisms. Fish densities of less than 35,000/m^3 should result in reduced cannibalism.

Foods

Effective first feeding may be the greatest problem in the intensive culture of larval walleyes. Walleyes accept live foods more readily than formulated diets, and size-graded zooplankton or brine shrimp nauplii are the recommended starter foods. Cannibalism is a significant problem during the first 2 weeks of feeding and may result from a combination of predatory behavior, fish density, and food size, type, and availability (Olson 1974; Cuff 1977, 1980; Nickum 1978; Beyerle 1979).

The use of formulated diets as starter feed has not been successful. Fish may eat the food but die after a few weeks, suffering from what Nickum (1978) described as the "dwindles." Foods eaten by first-feeding larvae range in length from 0.2 mm for dry particles to 1.5 mm for *Cyclops* copepods. Particles 0.3–0.4 mm long are satisfactory for first-feeding walleyes (Merna 1977; McElman and Balon 1979), although Nickum (1978) suggested slightly smaller sizes of 0.2–0.25 mm. Larger size copepods 0.9–1.5 mm are also recommended (Mathias and Li 1982; Raisanen and Applegate 1983) (Table 3).

There are two methods of providing larval walleyes with live food. First is the use of collected (not intensively cultured) zooplankton or cultured freshwater plankton such as *Daphnia*. The second comprises feeding brine shrimp, which are commercially available as eggs or cysts. Feeding techniques are discussed in a separate section of this paper.

Zooplankton harvested from ponds usually includes many of the species of organisms eaten by wild walleyes and those reared extensively in ponds. Results of several food preference studies indicate a selectivity for prey that changes as walleye size increases. Larvae less than 13 mm long select copepods such as *Diaptomus* and *Cyclops* (Houde 1967; Priegel 1970; Bulkley et al. 1976; McWilliams 1976) or small cladocerans such as *Bosmina* (Spykerman 1974). Larvae larger than 13 mm select *Leptodora* (Priegel 1970), *Epischura* (Houde 1967), or *Daphnia* (McWilliams 1976). Daphnids are often the most abundant plankton species; however, walleyes usually select other species in spite of this (Houde 1967; Priegel 1970; Spykerman 1974).

Raisanen and Applegate (1983), using zooplankton collected from sewage lagoons, determined that walleyes selected *Moina brachiata* over *Daphnia, Cyclops, Asplanchna,* and *Brachionus*. *Cyclops* were consumed according to their concentration, or were preferred to other organisms when *Moina* was unavailable. Mathias and Li (1982) observed that walleyes fed on adult instars of *Daphnia pulex* or *Cyclops bicuspidatus,* rather than rotifers and copepod nauplii, in quantities equal to their concentration in the environment. They also noted that walleyes consumed prey of progressively increasing size as the fish grew, although the selection was less pronounced with *Daphnia*—due possibly to its round body shape. Using *Daphnia,* Mathias and Li (1982) also noted that 20-mm-long walleyes selected the smallest available organisms, those roughly 1.1–1.5 mm long and rejected *Daphnia* longer than 2.0 mm.

Freshwater zooplankton used in fish culture are either netted from lakes, streams, or reservoirs, or are grown in fertilized ponds; of these, daphnids are the most common organisms grown. In any case, food organisms should be graded so that fish are fed appropriately sized plankton.

A number of problems are inherent in the use of collected zooplankton as food. First, the desired species may not be available. If walleye eggs are hatched and reared in heated water, plankton blooms in lakes may lag behind fish production. Second, capture rates are often erratic and capture in the wild may not provide sufficient or reliable quantities of properly sized organisms. Third, the time cost may be prohibitive, especially at understaffed hatcheries. Fourth, grading and sorting equipment is necessary to ensure that walleye larvae receive suitably sized organisms. Some of these difficulties, however, can be overcome through the use of brine shrimp.

The use of brine shrimp nauplii also requires specialized equipment and techniques. Brine shrimp eggs, purchased in bulk, are incubated in a salt solution and hatch in roughly 24 h. One common hatching method uses fish-egg incubation jars contained in a heated and lighted cabinet (Meade et al. 1980). Decapsulation, or removal of the outer chorion with a chlorine solution (Bruggeman et al. 1977; Sorgeloos et al. 1977; Haines et al. 1980), increases egg viability (Bruggeman et al. 1977).

Survival time of brine shrimp in fresh water is limited, and the daily ration must be added in frequent feedings to provide live food throughout the day. Theis and Howey (1980) described an electronically controlled feeder that maintains and dispenses live food. Tank cleaning may become a problem when brine shrimp are used because mats of uneaten food form on the tank bottom. Unless unhatched eggs are removed they will add to the debris that must be cleaned. As fish larvae grow, they need food larger than newly hatched brine shrimp nauplii, or losses will occur. Brine shrimp, cultured to larger sizes, or freshwater plankton must be used for food once larvae become larger than 12–15 mm.

Feeding

Extensive monitoring of larval development may not be necessary if TU guidelines for culture are established and closely followed. However, the culturist should know how quickly the particular strain of fish develops at a specific rearing facility. In general, feeding practices involve feeding to excess; consequently, feeding-rate ranges have not been well established. Natural plankton has been fed as it is available, and it is most often ungraded for size or species. Suggested feeding rates with brine shrimp, expressed as number per fish per day, have ranged from 100 (Colesante and Youmans 1981) to 1,100 (Howey et al. 1980). By using 15 μg as the wet weight of individual brine shrimp (Bryant and Matty 1980) and 3.2 mg as the weight of individual fish at first feeding (Wiggins et al. 1981), one derives a daily feeding rate of 50 to 550% of body weight (BW). A suggested feeding regime, in which brine shrimp are the primary

TABLE 4.—Daily feeding regime for walleye fry. Gram or percent body weight values refer to wet weights.

Age post-hatch (d)	Food organism	Food size	Food amount per 20,000 fry	Minimum food as % body weight per day
0–3	Zooplankton in pond water	200 μm	At least 100 g of zooplankton	250
3–12	Brine shrimp nauplii	200–250 μm	60–90 mL of hatched cysts, or at least 110 g	215
8–12	Supplement brine shrimp feedings with natural zooplankton or brine shrimp 1–3 mm long	200–300 μm	As consumed (in addition to 110 g of nauplii above)	
12–20	Brine shrimp 1–4 mm long	200–350 μm	At least 115 g	190
20–30	Brine shrimp	200–400 μm	36 mL of hatched brine shrimp cysts	150
	Daphnia	400–600 μm	As consumed	
	Large brine shrimp	4 mm or longer	As consumed or at least 120 g	

food source, is given in Table 4. Comparison of a brine shrimp feeding regime with one of mixed zooplankton reveals the magnitude of wasted brine shrimp. Mathias and Li (1982) recommended zooplankton densities of 100–200 animals per liter in the fish culture tank; they established a feeding rate of live prey of 85% BW for walleyes 19 mm long. Smaller larval fish would presumably require amounts exceeding 85% BW.

In intensive culture studies with larval fish other than walleyes, the larvae are provided with a continuous minimum density of food organisms rather than with a daily amount of food based on a percentage of their body weight. Eldridge et al. (1981) reported that striped bass require a prey density of 0.1 organism/mL for normal growth. For larval Atlantic herring (*Clupea harengus*), Werner and Blaxter (1980) determined that a prey density of *Artemia* near 0.17/mL represented a threshold above which changes in prey numbers had little effect on survival or growth. Li and Mathias (1982) found similar results with larval walleyes, stating that densities of daphnids should be in the range of 0.1 to 0.2/mL. Prey density is probably a much better indicator of food availability than amount of food offered per unit of time.

Although it is important to provide an ample amount of food, the minimum feeding rate for walleye fry depends on availability of the food to the fry. Nickum (1978) suggested feeding formulated diets every 5 min, while Wiggins et al. (1981) fed formulated diets to fry every 2.5 min and brine shrimp every 5 min. The longest reported interval between feedings was 30 min (Howey et al. 1980). A continuously available food supply may be essential for establishing initial feeding and, later, for reducing cannibalism.

Summary

Development of an intensive-culture strategy for walleye requires the coordination of environment, food, and feeding techniques with larval development. Some of the most important factors affecting larval walleye survival are culture environment, water temperature, food characteristics, timing of feeding, food density, and fish density. Cannibalism can be reduced, in part, by adjustments of fish density, food density, and timing of first feeding.

Based on this literature review, some recommendations for intensive culture of larval walleyes are: (1) select gray culture tanks or tanks of a color that will provide contrast between water and food; (2) use water temperatures of 18–24°C; (3) provide uniform overhead lighting for the entire tank—do not cover part of the culture tank; (4) stock embryos at a rate of 35,000/m^3 or less in tanks larger than 100 L capacity; (5) monitor larval development to assure timely introduction of food; (6) use live food, either natural zooplankton, or brine shrimp, or a combination of the two, and provide the first feeding just prior to yolk absorption (100 TU); (7) maintain a food density of at least 0.1 organism/mL in culture tanks, 24 h/d; and (8) provide properly sized food of preferred plankton species (Table 3).

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Revised Estimates of Growth Requirements and Lethal Temperature Limits of Juvenile Walleyes

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Abstract.—The optimum temperature for growth of juvenile walleyes (*Stizostedion vitreum*) was estimated by rearing fish under a low light intensity (i.e., 5 lx). The upper lethal temperature was estimated by slowly raising the acclimation temperature 0.5°C/d until death occurred. The estimates of 26°C and 34.1°C, respectively, were similar to those observed for juvenile walleyes in outdoor experimental channels, but were higher than those determined in the laboratory by one of the present authors for Minnesota walleyes. Some possible sources of error include the light intensity differences between experiments, which can influence predator–prey interactions (ration levels) in laboratory tanks, and the methods of fish acclimation to lethal temperature.

Knowledge of accurate thermal requirements of freshwater fishes is required for fish hatcheries, for use in laboratory experiments, and for water quality management. The best available information on the temperature requirements for growth, survival, reproduction, and preferred temperatures of freshwater and marine fishes has been compiled by the National Academy of Sciences and National Academy of Engineering (1973) and the USEPA (1976). Little is known about the sources of error in temperature bioassay, and accuracy of test results is left to intuitive arguments in lieu of field validation. Discrepancies in thermal requirements of 2–4°C are important to electric generating utilities keeping down the costs of waste management. Therefore, we need field validation to measure accuracy of our laboratory results and to make improvements in bioassay methods. Hokanson (1977) observed that the estimated lethal temperatures have varied as much as 4–6°C among duplicate tests on the same species and growth optima can vary 2–7°C (see also Koenst and Smith 1982).

Smith and Koenst (1975) reported an incipient lethal temperature for juvenile walleyes (*Stizostedion vitreum*) of 31.6°C, an optimum temperature for growth of 22°C, and a zero net growth at 29°C. In experimental stream channels, total mortality of this species did not occur until water temperatures exceeded 34°C and positive growth was observed above 29°C (Wrenn and Forsythe 1978).

The purpose of our study was to estimate the optimum temperature for growth of juvenile walleyes when reared under a low light intensity; to revise the upper lethal temperature when acclimation temperature was slowly increased to the death point; to explain these differences between laboratory and field experiments; and to develop laboratory procedures to duplicate the field response.

Methods

The water, supplied from a deep well, flowed continuously through polyvinyl chloride pipes into each head tank. The water quality of the well water was reported by Smith et al. (1976); the test conditions are reported in Table 1.

All tests were conducted in 210 × 54 × 54-cm fiberglass tanks with a 30-cm standpipe to maintain a volume of 340 L. The water in each experimental tank was supplied from its own head tank, where dissolved oxygen and temperature were individually regulated. Water temperatures in each head tank were regulated by either electrical immersion heaters (Smith and Koenst 1975) or a thermostatically controlled solenoid valve that allowed hot water to flow through a series of immersed stainless steel heating coils. Water temperature was measured continuously by thermograph and daily readings were made by an immersion thermometer graduated by 0.1°C. Water temperatures fluctuated slightly (e.g., 1 SD ranged from 0.05 to 0.20°C). Airstones were used to maintain dissolved oxygen concentrations near air saturation both in the head tanks and in the experimental tanks. Water flowed by gravity from the head tank through a rubber garden hose to a hor-

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TABLE 1.—Water quality conditions in walleye study.

Item	Range or mean
Flow rate (L/min)	1.8–2.0
Dissolved oxygen[a] (% air saturation)	81–90
pH[b]	8.2–8.3
Alkalinity (mg/L as $CaCO_3$)	235

[a] Measured twice weekly.
[b] Measured weekly.

izontally placed polyvinyl chloride pipe with three constricted glass outlet tubes placed equidistant above each fish tank. Day length was maintained at a 15-h-light : 9-h-dark photoperiod during acclimation and testing. Two-thirds of each experimental tank was covered with black plastic to reduce light intensity. The unshaded portion of each experimental tank was illuminated by a 60-W incandescent bulb (5.4 lx) during the 2-week experimental period.

Pond-reared juvenile walleyes were obtained from the Minnesota Department of Natural Resources in October 1978. Fish were kept in holding tanks at 11°C prior to acclimation. Fish were subsequently acclimated at the rate of 1°C/d and held at 22°C until they were transferred to the experimental tanks. The fish were fed excess rations of fathead minnows (*Pimephales promelas*), one-seventh the body length of the predator, throughout the acclimation and testing periods. Upon arrival at the laboratory, all fish were given a prophylactic treatment of formalin plus malachite green oxalate for 3 d (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975).

In mid-January, after screening for uniform size (mean wet weight, 26.6 g; range, 18.9–39.4 g), 10 fish were randomly assigned to each of eight experimental tanks. Fish were acclimated to test temperatures (16–30°C) at the rate of 1°C/d and were held for an additional 3 weeks after the final test temperatures were reached. All fish were anesthetized with tricaine (MS-222) and cold-branded to mark individuals. The numerical brand was placed dorsally above the base of the pectoral fin. Fish were blotted with paper towels and weighed to the nearest 0.01 g and measured to the nearest millimeter during the marking procedures. Fish were measured again after a 2-week acclimation period and after a 2-week experimental period. Mortalities were recorded daily during the acclimation and testing periods. Growth over 2-week intervals was expressed as a specific rate (percent change in weight per time) after Brett et al. (1969). Growth rates for individual fish within a tank were averaged, and a cubic equation was fitted to our data to describe the growth rate as a function of temperature. This equation was chosen because it was not believed that a quadratic equation was adequate over the range of temperatures tested. The 95% confidence intervals around the fitted line were calculated and plotted to provide a visual description of the accuracy of the fitted line. The R^2, adjusted for degrees of freedom, is used to describe the goodness of fit of the equation.

The ultimate upper incipient lethal temperature (UUILT) is the highest incipient lethal temperature (i.e., median lethal dose) to which fish can attain by extreme thermal acclimation (Fry 1971). The UUILT was determined by exposing acclimated fish (i.e., fish reared at constant temperatures near 22, 26, and 28°C in the growth study) to a slow temperature rise (0.5°C/d) until death, using the methods of Cocking (1959). Fish were not fed at temperatures above 30°C. Graphical interpolation of the percent survival and the corresponding mean daily temperature in the preceding and final 24-h interval was used to determine the temperature at which 50% of the population would die.

Results and Discussion

Growth rates increased with increasing temperature to 26°C reaching a maximum of 2.11%/d (Figure 1). The optimum range for growth (i.e., temperature range that supported at least 80% of maximum growth) was 22–28°C. Growth rates at higher temperatures declined with increasing temperature. The line of best fit is described by the equation

$$G = 1.98 + 0.117X + 0.0218X^2 + 0.0017X^3; \qquad (1)$$

G = specific growth rate;
X = water temperature − 23°C.

The adjusted R^2 for this equation was 85.0%. A positive growth rate of 1.06%/d was observed at 29.9°C, indicating that juvenile walleyes are capable of growing at temperatures above 30°C under these experimental conditions. No mortality occurred in the growth study. The optimum temperature for growth for juvenile walleyes in this study is similar to that observed for juvenile yellow perch (*Perca flavescens*, 28.0°C: McCormick 1976) and pikeperch (*Stizostedion lucioperca*, 28–30°C: Willemsen 1978).

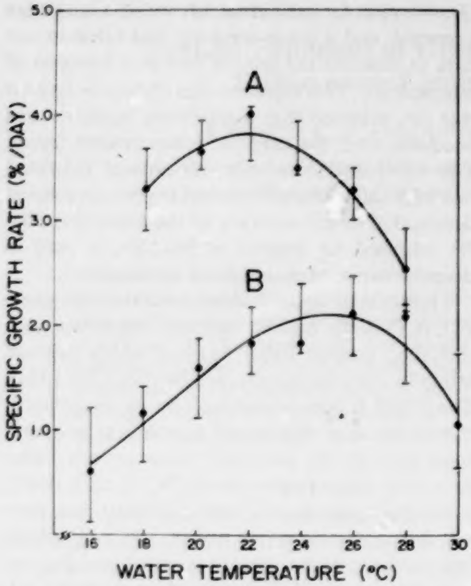

FIGURE 1.—Effects of water temperature on the specific growth rate of juvenile walleyes; observed data points and 95% confidence intervals of predicted values based on the regression equations. A, results obtained by Smith and Koenst (1975); B, present study.

TABLE 2.—The ultimate upper incipient lethal temperature of juvenile walleye.

Acclimation temperature (°C)	Highest incipient lethal temperature (°C)
22.1[a]+0.5°C/d	33.0
26.0[a]+0.5°C/d	34.1
28.0[a]+0.5°C/d	34.1
25.8[b]	31.6

[a] Temperature was constant for 49 d, then increased at the indicated rate; fish were not handled.

[b] Temperature was constant for 42 d, then fish were transferred to lethal-temperature baths (Smith and Koenst 1975).

Our results differ from those described by Smith and Koenst (1975) for juvenile walleyes (Figure 1). The line of best fit that describes their data is

$$G = 3.77 - 0.0723X - 0.0346X^2; \qquad (2)$$

G = specific growth rate;
X = water temperature − 23°C.

The adjusted R^2 for this equation was 88.2%. The quadratic is used to describe their data (equation 2) because their degrees of freedom were fewer and their temperature range was smaller than ours (equation 1). They reported a maximum specific growth rate of 4.007%/d and an optimum temperature of 22.1°C. However, they observed a maximum growth rate of 5.950%/d at the highest temperature tested (25.2°C) in their 1971 tests.

The UUILT was estimated for fish that survived the growth study at 22, 26, and 28°C. Raising the acclimation temperature slowly (0.5°C/d) to the death point increased the estimate of the UUILT of juvenile walleyes to 34.1°C (Table 2). The highest response was observed for an initial acclimation temperature of 26.0°C. Smith and Koenst (1975) reported an upper incipient lethal temperature (UILT) of 31.6°C for an acclimation temperature of 25.8°C using classical methods for temperature bioassay (Fry 1971). At rates of temperature increase of less than 1°C/d, fish acclimated more completely and gave a maximal response (Brett 1946; Cocking 1959). Fry acknowledged that this procedure can be used to estimate the UUILT, but notes its limitations for physiological analyses. The UUILT of juvenile walleyes (34.1°C) established in this study is similar to that of the juvenile yellow perch (32–34°C: McCormick 1976) and pikeperch (35.4°C: Willemsen 1978). It is noteworthy that these authors used methods similar to ours to raise the acclimation temperature of fish to its maximum.

The growth requirements and lethal temperature limits of juvenile walleyes established in this laboratory study support the findings of Wrenn and Forsythe (1978) under experimental field conditions. These results differ substantially from the findings of Smith and Koenst (1975) for this life phase and species. Since both studies were conducted by the same person (Koenst) in the laboratory using essentially Minnesota walleyes, the differences in results between studies seem to be due to differences in methodology in the respective tests. Some possible sources of error are discussed next.

These results suggest that, in laboratory experiments, light intensity must be carefully controlled for a species with known nocturnal activity patterns. The downward shift in the optimum temperature for walleye growth (Figure 1) is similar to what one might expect from restricted rations. Brett et al. (1969) observed an optimum temperature for growth of juvenile sockeye salmon (*Oncorhynchus nerka*) of 15°C when the fish were fed excess rations; the optimum temperature for growth declined to progressively lower levels for a corresponding decrease in ration level. Several differences in methods may have contributed to inferred lower rations in the study conducted by

Smith and Koenst (1975). In our study, fish were over 140 mm long and were reared at less than 5 lx. In the study conducted by Smith and Koenst (1975), light intensity over the open tanks was 230 lx and their fish measured over 63 mm in total length. The prey was of similar size in both studies and may have been more easily captured by the larger walleyes at the lower light intensities in our study. Juvenile walleyes in ponds are known to feed upon larger prey, including fish, more efficiently at nighttime (Mathias and Li 1982). Smith and Koenst used a smaller tank size (25 L) than we did (340 L). The interaction of these factors may have been responsible for the lower optimum growth temperature in the study conducted by Smith and Koenst (1975). Size differences in the fish used in these two studies may account for the differences in maximum growth rates (Figure 1), but are unlikely to account for the shift in the optimum temperature (Brett and Shelbourn 1975). Another species with nocturnal activity patterns, the white sucker (*Catostomus commersoni*), had higher food consumption, growth rates, and optimum temperature for growth when reared at lower than at higher light intensity (Koenst and Smith 1982).

Studies conducted by the same investigators with essentially the same stocks reveal that the methods used for acclimation to lethal temperature affect the fishes' response. McCauley and Binkowski (1982) estimated an UILT of 28.2°C for adult alewives acclimated to 27°C. Some nonthermal stress may have occurred because four of 10 fish died in the test bath at the acclimation temperature whereas none died in the acclimation tank itself. Handling and confinement in small tanks likely play an important role in lethal temperature tests of alewives. The unused fish (51) were kept 3 weeks at 30°C. No mortality occurred when the tank temperature was raised 1°C/d until 34°C was reached, then four fish died. The remainder died during the interval 34–35°C. Koenst and Smith (1982) estimated an UILT of 30.5°C for juvenile white suckers by transferring them directly from an acclimation temperature of 24.1°C to a series of lethal temperatures. They estimated an UUILT of juvenile white suckers of 32.5°C without handling them and by slowly increasing the acclimation temperature 0.5°C/d to the death point. The methods used to estimate an UILT and UUILT in both studies differed only in the procedures used to adjust the fish to lethal temperature. Indeed all the precautions in measurement of standard metabolism are needed to minimize the influence of accessory lethal factors and to estimate the UUILT. This has been achieved by the gradual acclimation procedure used in this study and these results have been verified by field evidence.

Acknowledgments

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Increased Walleye Egg Hatch and Larval Survival after Protease Treatment of Eggs

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Abstract.—Various techniques for eliminating the clumping of newly spawned walleye eggs (*Stizostedion vitreum*) were evaluated by measuring hatch, growth, and survival. Percent hatch was highest (83%) in egg groups water hardened in 0.01% protease solution. Other water-hardening techniques tested were: (1) continuous stirring; (2) 250 mg/L tannic acid solution; and (3) bentonite clay suspension. Low hatching percentages for eggs continuously stirred or treated with bentonite were attributed to fungus. Fungus was minimal on eggs treated with protease or tannic acid. Fish hatched from each egg treatment group were intensively cultured for 21 d in one of four rearing environments: (1) well water; (2) well water receiving 1% sea salt for the initial 3 d; (3) well water receiving a 6-mL daily addition of antifoam emulsion for the initial 9 d; or (4) well water receiving both the 1% sea salt and antifoam treatments. There was slightly higher survival in larval fish hatched from eggs treated with protease or tannic acid and reared in well water containing 1% sea salt. We observed no differences in average fish length among the test groups.

The success of hatchery production programs begins with the acquisition of suitable quality brood fish, and sufficient egg production. Also, maximum benefit must be derived from the egg source. The adhesiveness of walleye eggs (*Stizostedion vitreum*) results in clumping, which leads to handling problems and reduces access to oxygenated water during incubation; egg losses often are incurred. Three techniques are now commonly used to eliminate walleye egg adhesiveness during spawning and water-hardening procedures: stirring with a large turkey feather for 1.5–2 h after fertilization (Z. Bean, Pennsylvania Fish Commission, personal communication); physical scouring with abrasive particles (Hurley 1972; Waltemyer 1976); and chemical treatment with 285–400 mg/L tannic acid (Dumas and Brand 1972; Colesante and Youmans 1983). We compared the effectiveness of these three techniques with a fourth technique—exposure to protease enzyme. In Europe, protease has been used to separate catfish eggs (*Silurus* sp.; Horvath 1980) after spawning and during incubation. Our objective was to evaluate these methods by comparing hatching percentage and the survival and growth of larval fish from each egg treatment. These fish were then reared for 21 d in: (1) well water receiving 1% sea salt for the initial 3 d; (2) well water receiving a 6-mL daily addition of antifoam emulsion for the initial 9 d; (3) well water containing both 1% sea salt and antifoam additions; or (4) untreated well water.

Walleye organ systems develop and begin to function after hatching and before first feeding (Reighard 1890; McElman and Balon 1979). During this time, development of functional gills takes place and the circulatory pattern changes—the site of blood oxygenation moves from the ventral abdominal surface to the gills (McElman and Balon 1979). The fish are susceptible to stress caused by poor environmental conditions. Walleyes require water that has a high dissolved oxygen content. The embryos suspend themselves with their head up and their oil globule at the surface of the water. Because surface oil films may inhibit free gas exchange and decrease the fish's chance for survival, we added antifoam agent to reduce surface tension and disperse the surface oils. One percent sea salt was added to the rearing water to determine its value in reducing stress, enhancing ion regulation, and increasing the fish's buoyancy to reduce the energy required to maintain a position at the surface.

Methods

Wild brood fish were captured from Pymatuning Reservoir and transferred to Linesville Fish Production station, Pennsylvania Fish Commission. Water-hardening procedures were evaluated during spawning operations. A pretest was conducted to determine the lowest protease concentration that eliminated egg clumping in 30 min. Eggs from 9–11 ripe females were divided into two, then into four equal groups of 1 L each and transferred to dry pans; 2.0 mL of combined milt from two males was added to each pan. Eggs and sperm were mixed and 1 L of distilled water was added. Examination of eggs with a dissecting scope indicated that the micropyle closed after 1 min. Treatments were administered 3 min after micropyle closure to minimize direct entrance of protease into the egg. Protease (Sigma Chemical Company Type I, Pancreatic, Crude Activity 8–10 units/mg) in concentrations of 0.1, 0.01, 0.001, or 0.0001% was added. The solution was stirred continuously for 5 min, then stirred for 1 min at 5-min intervals thereafter. After 30 min the solution was poured off and 1 L of fresh distilled water was added. The stir and rinse procedure was repeated two more times with lake water, yielding a total treatment period of 90 min. The lowest concentration of protease causing eggs to separate was 0.01%.

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In the egg-treatment test, 0.01% protease was compared with bentonite suspension, 166 g/L H_2O; tannic acid solution, 250 mg/L; and 90 min continuous stirring (no additives). All treatments were repeated in three separate tests (total of 12 groups) on the same day. The fish were spawned and eggs fertilized as described in the protease pretest. Protease, tannic acid, and bentonite were added 4 min after fertilization. Specific treatment regimes follow.

Tannic acid solution was added 4 min after water was added to the fertilized eggs; the mixture was stirred for 4 min and then eggs were rinsed three times. After the initial addition of sperm, water was changed three times at 30-min intervals—a total of 90 min.

Protease solution was added 4 min after water was introduced to the fertilized eggs, followed by continuous stirring for 5 min. Eggs were stirred for 1 min at 5-min intervals for the next 30 min. Water was changed as in the tannic acid treatment.

Bentonite solution was added 4 min after water was introduced to the fertilized eggs, followed by continuous stirring for 10 min. Water was changed as in the tannic acid treatment.

Continuous stirring of eggs was carried out with a large turkey feather for 90 min. Water in the treatment pan was changed every 30 min.

After treatment, the eggs were maintained in separate flow-through buckets before they were transported for about 4 h to the National Fishery Research and Development Laboratory. The 12 lots of eggs were incubated, under identical conditions, in separate 6-L jars (May-Sloan, Incorporated) containing well water at temperatures ranging from 7 to 15°C. Fertilization and incubation were carried out in the optimum temperature ranges described by Smith and Koenst (1975). A rising temperature regime was established during incubation to optimize numbers of swim-up larvae and to reduce potential abnormality (Hokanson 1977). Every third day the eggs received a formalin treatment (1:6,000) for 15 min. On the fifth and sixth days, dead eggs and those infected with fungus were removed from the jars. Hatching began on the 10th day and was nearly complete by the end of the 11th day. On the 11th day, fish receiving the same egg treatments were pooled and randomly distributed to eight larval rearing tanks (approximately 5,000 7-mm fish per tank). There were four rearing environments: (1) two tanks received well water plus 1% sea salt for the initial 3 d; (2) two tanks received well water with 1 mL of antifoam emulsion (Sigma Chemical Company) added six times over 8 h each day for the first 9 d; (3) two tanks received well water containing a combination of the 1% sea salt and antifoam emulsion treatment; and (4) two control tanks received untreated well water. Water temperature was 18°C for all treatment groups.

Tanks were fiberglass, 275 × 60 cm, with inflow and feeding modifications for rearing larval walleyes (Figure 1). Each tank was fitted with controlled-delivery airlines, a live-feed feeder (Theis and Howey 1980), and a water inflow through a feed dispersal trough. Saran screen (300-μm mesh) was glued with silicone to stainless steel screen and fitted on the outflow of the tanks. Water depth was 15 cm. A solution of Ocean 50 seamix (Jungle Laboratories Corporation) was dispensed into culture tanks to maintain a 1% sea salt solution. The solution was dispensed from a hopper operated by a timed solenoid valve. Fish began feeding on the fifth day after hatching. They were fed brine shrimp nauplii (*Artemia* sp.) hatched in 6-L jars (Meade et al. 1980). Feeding rates were a minimum of 100% of body weight per day (wet weight of brine shrimp). Fish received feed at 10-min intervals, 14–16 h/d. All excess feed, unhatched cysts, and dead fish were siphoned off daily. Dead fish were hand counted daily and recorded. Larval fish were counted and their length and weight measured at the end of the test.

We used two-way analysis of variance and the Newman–Keuls test (Zar 1974) to determine statistical differences in percent egg hatch among egg treatments and two-way analysis of variance to determine statistical differences in fry survival

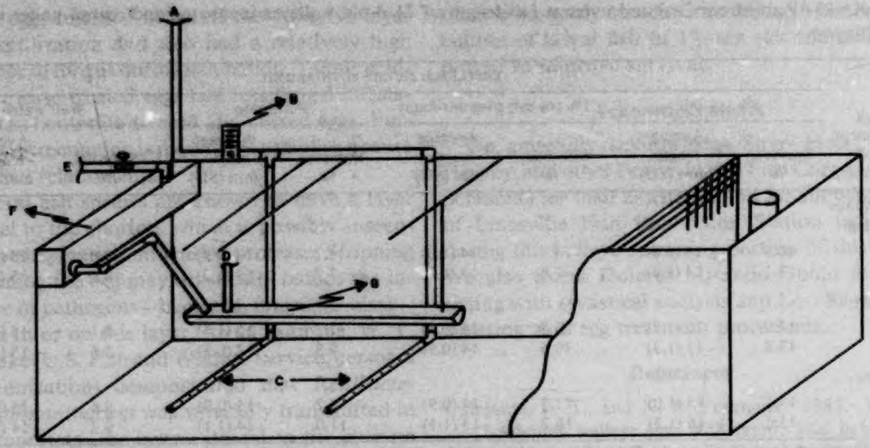

FIGURE 1.—Diagram of a tank used for feeding and rearing larval walleyes. A = air supply; B = air flowmeter and valve; C = air diffusers; D = feed inflow pipe to dispersal trough; E = water inflow; F = inflow head box; and G = mixing and dispersal trough.

among egg and larval treatments. Percentage means were transformed into their arcsine in order to change the binomial distribution of percentages to a normal distribution (Zar 1974).

Results

After the eggs were fertilized, their properties differed, depending on egg treatment. During the 4-min pretreatment phase, with the exception of the continuously stirred groups, all eggs began to stick to the spawning pan or clump slightly. During the following 90-min-treatment phase, stirred eggs remained separated, bentonite-treated eggs remained slightly clumped, protease-treated eggs remained clumped for 15–25 min, and tannic acid-treated eggs showed little or no clumping. Neither protease- nor tannic acid-treated eggs clumped after the 90-min period, whereas both the bentonite and stirred groups reaggregated after completion of the procedure. Tannic acid-treated eggs began changing from cream color to dark gray in the holding buckets prior to transportation to the laboratory.

During incubation, groups of bentonite-treated and stirred eggs remained clumped until days 3 and 4, respectively. The clumps then began to roll gently in the incubation jars; free eggs rolled vigorously. Remnants of the adhesive layer were still microscopically visible on stirred eggs after 5 d of incubation. Protease- and tannic acid-treated eggs rolled evenly in the jars during incubation.

The eggs became eyed on day 8 and began to hatch on day 10. Significant amounts of fungus were present only on stirred and bentonite-treated eggs. All of the egg groups were hatched by the end of the following day except for the tannic acid group, which required two additional days to complete hatching. Many fish (estimated 15–20%) from the tannic acid group hatched with the egg shell lodged over the head and midsection; these fish had difficulty freeing themselves.

Eggs remained in incubation jars until hatching was completed. Application of two-way analysis of variance of arcsine-transformed data and Newman–Keuls multiple range test (Zar 1974) showed that percent hatch of protease-treated eggs was significantly higher ($P < 0.05$) than that of eggs in the other treatments (Table 1).

Final percent survival and fish lengths after 21 d are given for all treatment groups (Table 2). Data for larval fish from bentonite-treated eggs are omitted because twice the number of larval fish were accidentally stocked into those rearing tanks than in the others. Two-way analysis of variance of arcsine-transformed survival data of the re-

TABLE 1.—Percent hatch of walleye eggs by egg treatment.

Egg treatment	Percent hatch			
	Lot 1	Lot 2	Lot 3	Average
Protease	88	90	71	83
Tannic acid	74	52	65	64
Stirred	48	60	38	48
Bentonite	52	44	36	44

TABLE 2.—Percent survival and average fish length of 21-d-old walleyes incubated and reared under selected conditions.[a]

Egg treatment and replication	Larval fish culture environment							
	1% sea salt		1% sea salt plus antifoam		Antifoam		Well water	
	Survival, %	Average length, mm (SD)	Survival, %	Average length, mm (SD)	Survival, %	Average length, mm (SD)	Survival, %	Average length, mm (SD)
Continuous stirring								
1	10.9	14 (0.8)	4.6	14 (1.0)	8.7	14 (0.9)	10.6	15 (1.5)
2	7.1	14 (1.8)	7.8	13 (1.1)	7.7	14 (1.2)	16.0	15 (0.9)
Tannic acid								
1	14.0	15 (1.2)	8.8	14 (0.6)	7.2	15 (1.5)	8.1	15 (1.2)
2	13.8	15 (1.3)	10.6	14 (0.9)	9.8	13 (0.8)	5.6	15 (1.8)
Protease								
1	13.1	15 (1.0)	24.5	14 (0.9)	8.2	15 (0.9)	9.6	15 (0.8)
2	13.6	16 (1.3)	16.2	15 (1.1)	11.0	14 (1.1)	9.2	14 (0.8)

[a] Percent survival = 100(number of survivors after 21 d) ÷ (number of dead fish removed from tank + number of survivors after 21 d). Cannibalism was assumed to be equal among groups. Average lengths were based on measurements of 10 fish.

maining groups indicated a significant influence by egg treatment ($P < 0.05$), but no significant influence by larval fish rearing conditions. Survival was calculated with the formula

percent survival = 100(number of survivors after 21 d) ÷ (number of dead fish removed from the tank + the number of survivors after 21 d).

Survival was greatly influenced by interaction of the egg and the larval fish rearing treatments ($P < 0.05$). Survival was highest in larvae from protease-treated eggs that were reared in 1% sea salt or in 1% sea salt plus antifoam and in those from tannic acid-treated eggs reared in 1% sea salt. In all tests, fish length was not influenced by egg treatment or larval fish rearing treatment.

Discussion

The use of harsh techniques to eliminate egg adhesiveness may adversely affect egg quality. In these tests, egg treatments were administered as gently as possible. The protease level was determined by selecting a concentration that required a full 30-min exposure to affect clumping. Waltemyer (1976) demonstrated that 200 mg/L tannic acid applied for 2 min was 80% effective in removal of adhesiveness when eggs were stirred. We used 250 mg/L tannic acid for a 4-min treatment, which was similar to treatments of 300–400 mg/L for 2–3 min used by Colesante and Youmans (1983). (Final tannic acid concentrations used by Colesante and Youmans (1983) appear to be 285 mg/L and 380 mg/L because 1 L of 6,000- or 8,000-mg/L stock solution was added to 20 L of water, leaving a total liquid of 21 L in each tank.) The 250-mg/L treatment also eliminated the need to unclump eggs during the incubation process. Unclumping eggs with the initial egg treatment reduced problems with floating egg clumps and fungus infestation that occurred during incubation.

Manipulation of culture conditions may account for increases in larval fish survival. Increased salinity at low levels improves ionic conditions in soft water. Studies by Wedemeyer (1972) showed that addition of 100 mmol or more of NaCl prevented stress-induced blood hypochloremia in steelhead (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Redding and Schreck (1983) described a decrease in plasma osmolarity and electrolyte balance in stressed coho salmon held in fresh water. In the same test, coho salmon held in one-third concentration seawater suffered no osmotic imbalance. Maintaining walleye larvae in 1% salt concentrations resulted in the highest larval fish survival—results similar to those observed by Germann and Reeves (1975) with striped bass larvae (*Morone saxatilis*). Salts may also reduce fungal infestations in intensive culture, thereby decreasing chances of fungus-caused mortality.

The external sticky layer of walleye eggs may provide a nutrient base for bacteria and fungi. Microscopic observation revealed fungal hyphae that had spread to adjacent live eggs in clumps containing a few dead eggs. Bentonite-treated and stirred eggs retained much of the adhesive layer after fertilization and also had a relatively high incidence of fungus during incubation. Tannic acid- and protease-treated eggs had less fungal infestation than bentonite-treated and stirred eggs. Further experimentation is required to establish a cause and effect relationship.

Several fish species are known to have a layer external to the chorion, which is possibly susceptible to enzymatic removal by protease. Stripping or cleaning the egg may drastically reduce the incidence of pathogens—bacterial, fungal, or viral—carried in or on this layer. As an example, W. T. Yasutake (U.S. Fish and Wildlife Service, personal communication) demonstrated that *Renibacterium salmoninarum* was vertically transmitted in a mucoprotein-like layer external to the chorion in eggs of chinook salmon (*Oncorhynchus tshawytscha*).

High egg quality is of paramount importance, both to increase the percent hatch and to improve larval fish survival. Egg quality is measured by percent hatch, fish survival, and lack of anomalies caused by treatment. In each of our tests, protease-treated eggs yielded the highest survival. Tannic acid-treated eggs, although free of fungus, produced fewer fish. Fish had to free themselves from a hardened eggshell, usually lodged over the head. Energy cost of this activity and its effect on survival of larvae is unknown. Also, tannic acid-treated eggs required 4 d instead of 2 d to hatch. Such extension of the hatching period could result in a significant size difference 10 d after hatch, leaving small fish prey for cannibals. Protease and tannic acid treatments proved advantageous because handling time was reduced; neither stirring nor other care was necessary after treatment, although protease-treated eggs were stirred 1 min at 5-min intervals during treatment. Reduction of handling time to remove stickiness in eggs allows more time for other spawning or hatchery chores.

These results indicate that 0.01% protease solution can help increase hatchery efficiency and production. Efficiency is increased because protease quickly removes adhesiveness from walleye eggs, thus reducing egg handling labor while yielding a higher egg hatch. As percent hatch is increased, available fry numbers will increase accordingly. A 10% increase in hatch would add 100,000 more fish into a hatchery program for every 1,000,000 eggs available. Protease treatment may also be a method of controlling fungus. Survival of larval fish hatched from these eggs was higher than that of larval fish from eggs treated by other commonly standard methods. After hatch, culture of larval fish in 1% sea salt solution appeared to improve survival.

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Growth of Tiger Muskellunge Fed Different Amounts of Protein at Three Water Temperatures

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Abstract.—Growth rates of tiger muskellunge (muskellunge *Esox masquinongy* ♀ × northern pike *E. lucius* ♂) fed diets containing 35, 45, or 55% crude protein for 5 weeks at 17, 20, or 23°C were compared. Fish fed diets containing 45 or 55% protein grew faster at all temperatures than those fed 35% protein. Growth of tiger muskellunge fed a diet containing either 45 or 55% protein did not increase significantly at optimum growth temperatures (20 or 23°C). At 17°C, below the optimum temperature range, growth did increase when the percentage of protein in the diet was increased.

Diet and temperature are two of the most important factors controlling the growth of tiger muskellunge (muskellunge *Esox masquinongy* ♀ × northern pike *E. lucius* ♂) under intensive culture. Diets in current use produce acceptable growth but are high in energy and protein (>50%). Because protein is the most expensive component of a diet, knowledge about the protein requirements of the fish is essential for the development of nutritionally complete, economical feeds. Little is known about the nutritional requirements of coolwater fishes, but various studies have shown that the quantity of protein needed for optimum growth varies with species (DeLong et al. 1958; Ogino and Satio 1970; Cowey et al. 1972; Sabaut and Luquet 1973; Satia 1974; Davis and Stickney 1978) and temperature (DeLong et al. 1958; Andrews and Stickney 1972). In some studies, protein requirements of fish have been shown to change with size and age (Page and Andrews 1973; Satia 1974). The objectives in this study were to measure the growth, survival, and body composition of tiger muskellunge reared at 17, 20, or 23°C on diets containing 35, 45, or 55% protein. We examined tissues histologically to determine if these diets had any detrimental effects.

Methods

Tiger muskellunge fry, provided by the Pennsylvania Fish Commission, were fed U.S. Fish and Wildlife Service diet W-7 and were held at 20°C for the first 2 weeks of feeding until they had reached a size (about 50 mm long) at which cannibalism, a characteristic of this hybrid, became uncommon. Groups of 15 fish were randomly captured, weighed, and counted into each of 27 7-L cylindrical test jars. Each jar was supplied with 2 L/min fresh, aerated, heated well water: dissolved oxygen, 8.2–9.6 mg/L; pH, 7.3–7.5; total hardness, 30–40 mg/L as $CaCO_3$; and total dissolved gas saturation, 103%. Water inflow rates were adjusted weekly. Water temperatures were adjusted to 17, 20, or 23 ± 1°C and monitored daily.

Three diets were tested at the three water temperatures in triplicate replication. Water temperature and diet were randomly assigned to each jar. Fish were acclimated to test conditions for 2 weeks before the start of the study; during this time, cannibals and dead or missing fish were replaced with fish of about the same size. A cannibal was any tiger muskellunge with another fish in its mouth or stomach. Average initial fish weight was 0.71 g.

Three test diets, containing 35, 45, or 55% protein, were formulated by adjusting the amount of fish protein concentrate (Swedish Protein, Molndal, Sweden), gelatin, and dextrin in each mixture while maintaining the same lipid addition (Table 1). Calcium and phosphorus were added to adjust the concentrations in the test diets to 2.1% and 1.4%, respectively (diet ingredients entered into this calculation). Metabolizable energy content of the diets, calculated from nutritional information available for trout (Smith 1976), ranged from 3.5 to 3.8 kcal/g (Table 1). Calculated amino acid levels (percent of dietary protein) of all diets met or exceeded the requirements established for chinook salmon (*Oncorhynchus tshawytscha*: Mertz 1969).

Fish were fed 64 times per day with automatic feeders at 15-min intervals between 0400 and 2000 hours each day. The amount of food dispensed to each group was similar each day and was in excess of that typically given to tiger muskellunge (Meade et al. 1983). Jars and feeders were cleaned, dead fish and cannibals removed and weighed, and the feeders adjusted each day between 0800–1100 hours. Fish in each replicate were weighed in mass to the nearest 0.1 g in water each week. Slime and fungus were controlled by brushing jars with salt when the fish were removed for weighing and counting. Windows in the laboratory were covered with black plastic to exclude extraneous light. Overhead fluorescent lights were on between 0730 and 1630 hours and subdued indirect fluorescent lights were on during the remainder of the day. Light intensity at the water surface was 5–7 lx when overhead lights were on and 3 lx at other times.

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The study was terminated at 5 weeks when some fish became too large to be held in the jars. All fish were weighed individually to the nearest 0.1 g. Specific growth rates (SGR) of fish (expressed as percent weight gain per day) were calculated by the equation (Brown 1957)

$$SGR = (\log_e \text{final weight} - \log_e \text{beginning weight})/d.$$

Specific growth rates were computed to allow us to compare fish that had slightly different weights after the 2-week acclimation period.

Tissues of five fish from each diet group held at 20°C were examined for gross pathological and histological changes. The tissues were preserved in Bouin's solution, embedded in paraffin, cut to 4 μm thickness, and stained with hematoxylin and eosin. Proximate compositions of fish were determined from a composite sample of 200 fish collected at the beginning of the study and from samples of five fish from each jar collected at the end of the study. Feed was withheld from these fish for 24 h to ensure that the gut was empty before the samples were taken. Randomly sampled fish were killed with a lethal dose of anesthetic, weighed, and frozen. Frozen fish were chopped into 1–2-cm pieces and freeze-dried to measure moisture content. Dried fish were ground through a 20-gauge screen in a Wiley mill and subdivided into samples for protein, lipid, and ash analyses. Lipid content was measured in a Goldfisch lipid extraction apparatus after a 7-h extraction with chloroform–methanol (2:1). Protein and ash were determined according to standard procedures of the Association of Official Analytical Chemists (Horwitz 1975). Proximate compositions of fish were reported on a wet-weight basis.

TABLE 1.—Composition and energy content of test diets containing different amounts of protein.

Component	35% protein diet	45% protein diet	55% protein diet
	Diet composition (%)		
Fish protein[a]	37.5	48.0	59.1
Gelatin	5.7	7.0	8.6
Dextrin	33.3	22.2	11.3
Fish oil	14.0	14.0	14.0
Methionine	0.5	0.6	0.7
$CaCO_3$[b]	0.39	0.26	0.13
$CaHPO_4 \cdot 2H_2O$[b]	3.66	2.98	1.22
Vitamins[c]	1.1	1.1	1.1
Minerals[d]	3.8	3.8	3.8
	Proximate composition (%)		
Protein	35.59	45.46	55.15
Lipids	11.74	12.02	12.97
Moisture	9.09	8.98	8.21
Ash	9.87	10.66	10.85
Carbohydrate[e]	33.71	22.88	12.82
	Energy content (kcal/g)		
Gross[f]	4.4	4.5	4.7
Metabolizable[g]	3.5	3.5	3.8

[a] Swedish Protein AB, Box 264, S-43123, Molndal 1, Sweden: crude protein, 80%; fat, 0.3%; ash, 15%; moisture, 6%.

[b] Calcium and phosphorus added separately to adjust levels in diets to 2.1 and 1.4%, respectively.

[c] Vitamins (in mg/kg of feed unless otherwise specified, IU = International Units): thiamine HCl, 40; riboflavin, 30; D-calcium pantothenate, 150; niacinamide, 150; pyridoxine HCl, 20; D-biotin, 3; folic acid, 15; vitamin B_{12}, 0.2; inositol, 1,000; menadione, 10; ascorbic acid, 1,000; choline chloride (70%), 4,000; vitamin A palmitate, 10,000 IU; vitamin D_3, 4,000 IU; vitamin E, 646 IU; and ethoxyquin (antioxidant), 150.

[d] Minerals (in mg/kg of feed): $CuSO_4 \cdot 5H_2O$, 60; KIO_3, 9; $FeSO_4 \cdot 7H_2O$, 500; $MnSO_4 \cdot H_2O$, 315; $Na_2MoO_4 \cdot 2H_2O$, 8; $NaSeO_3$, 0.3; and $ZnSO_4 \cdot 7H_2O$, 680.

[e] Carbohydrate content was not measured but was determined by difference.

[f] Complete combustion in calorimeter.

[g] Calculated from metabolizable energy values of ingredients as determined for trout (Smith 1976).

Two-way analysis of variance (Sokal and Rohlf 1969) was used to compare growth and survival of fish fed different diets at various temperatures. Data were fitted to the second degree polynomial regression equation

$$Y = b_0 + b_1X + b_2X^2,$$

as described by Zeitoun et al. (1976), where Y is the percent weight gained in grams, X is the protein content of the diet in percentage, and b_0, b_1, and b_2 are empirically derived coefficients. This equation has been used for making economic decisions by relating fish protein requirements to maximum economic returns.

TABLE 2.—Comparison by two-way analyses of variance of growth and survival of tiger muskellunge held at different temperatures and fed different diets. Asterisk denotes significant difference at $P < 0.01$.

Analysis of variance	Degrees of freedom	F-ratio
Survival (%)		
Diet	2	0.66
Temperature	2	0.64
Interaction	4	0.62
Specific growth rate (%/d)		
Diet	2	68.40*
Temperature	2	13.34*
Interaction	4	6.69*

Results and Discussion

The nutritional requirements of tiger muskellunge, like those of many other coolwater fishes, are poorly known. The fish appear to eat and grow well, at least through the fingerling size, on available nutrient-dense (high-protein) diets, such as the U.S. Fish and Wildlife Service diet W-7. Early adaptation to these diets is essential in order to reduce cannibalism during the prefingerling periods of intensive culture.

Survival

Cannibalism by tiger muskellunge was not a problem after a 2-week acclimation period and few fish died (0–2 fish/jar) during the 5-week study. The effect of protein content of the diet on fish survival (Table 2) was not statistically significant ($P > 0.05$).

Temperature Effects

Specific growth rates of tiger muskellunge were significantly related to temperature and diet and to the interaction between these variables (Table 2). Maximum growth occurred when fish were fed rations containing at least 45% protein at temperatures between 20 and 23°C. This agrees with the observation of Meade et al. (1983) that the optimum temperature for growth and food-conversion efficiency of fry and fingerling tiger muskellunge is between 20 and 23°C. The reduced growth rate at 23°C for tiger muskellunge fed the diet lowest in protein suggests that, at this temperature, protein content can be limiting (Figure 1). This suggestion was supported by the observation that growth increased dramatically at 17°C when the protein level of the diet was increased (Figures 1, 2). Because proximate compositions were similar for all fish fed the same diet, regardless of temperature, data were combined for each diet (Table 3).

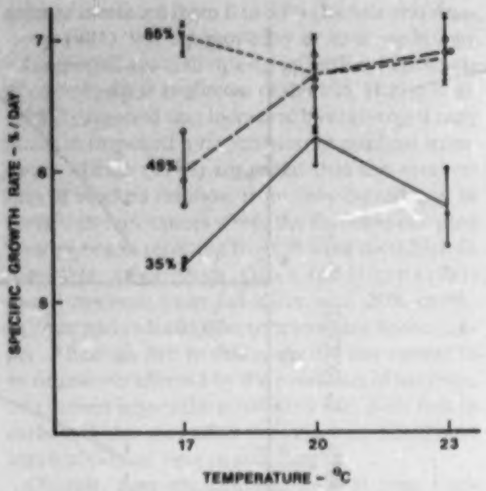

FIGURE 1.—Relation between water temperature and growth rate of tiger muskellunge fed diets containing 35, 45, or 55% protein. Vertical lines embrace the range of three replicates.

Dietary Protein Effects

Growth of fish increased with a change in dietary protein from 35 to 45% at all three temperatures; however, an additional 10% increase in the protein content of the diet did not result in further significant increases in growth except at 17°C. Growth rates were significantly less for all fish fed the 35% protein diet than for those fed the 45 or 55% protein diets, indicating that this diet contains insufficient protein to provide maximum growth at the temperatures tested (Figure 1).

The polynomial growth curves fitted to the percentage weight gain of fish fed the 35, 45 or 55% protein test diets at 17, 20, or 23°C revealed that, at 20 and 23°C, growth of tiger muskellunge 3–13 cm long was fastest when they were fed diets containing about 50% protein (Figure 2). At 17°C, however, the data did not conform to the pattern observed at 20 and 23°C; they suggest that, had we tested higher protein diets, the curve for fish at 17°C would probably have conformed more closely to that of fish held at higher temperatures. This would have allowed us to clearly define the point of maximum growth at 17°C. Overall, temperature is still the controlling factor in the growth response of fish to dietary levels of protein. Meade et al. (1983) observed that the early specific growth

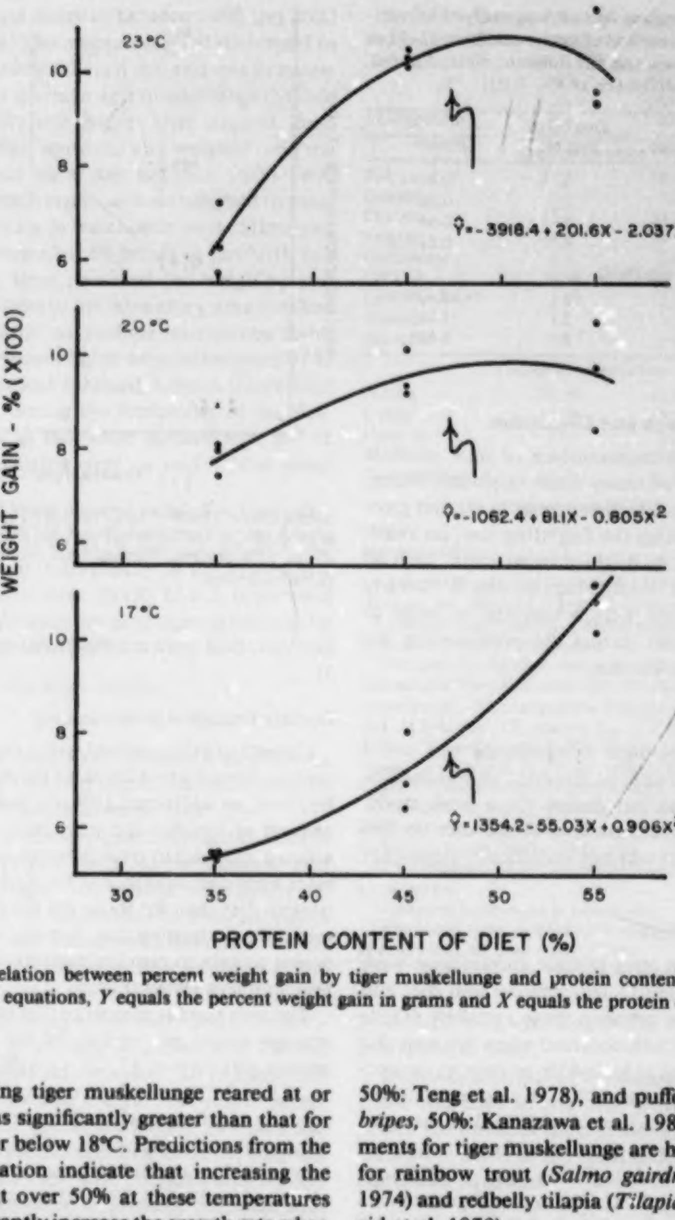

FIGURE 2.—Relation between percent weight gain by tiger muskellunge and protein content of diets at three temperatures. In equations, *Y* equals the percent weight gain in grams and *X* equals the protein content of the diet in percent.

rate of fingerling tiger muskellunge reared at or above 20°C was significantly greater than that for fish reared at or below 18°C. Predictions from the regression equation indicate that increasing the protein content over 50% at these temperatures will not significantly increase the growth rate when the main protein source is fish protein concentrate. The protein requirements for tiger muskellunge are similar to those reported for smallmouth bass (*Micropterus dolomieui*, 45%: Anderson et al. 1981), chinook salmon (40–55%: Delong et al. 1958), plaice (*Pleuronectes platessa*, 50%: Cowey et al. 1972), grouper (*Epinephelus salmoides*, 40–50%: Teng et al. 1978), and puffer fish (*Fugu rubripes*, 50%: Kanazawa et al. 1980). The requirements for tiger muskellunge are higher than those for rainbow trout (*Salmo gairdneri*, 40%: Satia 1974) and redbelly tilapia (*Tilapia zilli*, 35%: Mazid et al. 1979).

One of the dilemmas of diet testing is that at least one other component of a diet must be changed in order to vary the concentration of the component to be tested. The part of a diet most often varied, but usually not tested, is the carbohydrate component. Dextrin, the digestible carbohydrate used to make diets essentially equal in

TABLE 3.—Proximate composition of tiger muskellunge at the end of the 5-week study. Each value is a mean of nine values.

Protein content of diets (%)	Proximate composition (%, wet weight basis)[a,b]			
	Protein	Lipid	Ash	Moisture
35	14.4	3.7	2.6	78.8
45	14.5	3.8	2.6	78.8
55	15.0	3.4	2.5	78.7
Pooled SE	0.236	0.110	0.073	0.332

[a] Composition of fish at beginning of study (wet weight basis): protein, 11.7%; lipid, 2.8%; ash, 1.7%; and moisture, 83.7%.

[b] No significant differences ($P > 0.05$) among proximate compositions of fish fed diets containing different amounts of protein.

energy and lipid content, was present in highest percentage in the diet that contained the lowest amount of protein and produced the slowest growth rate of tiger muskellunge; the 35, 45, and 55% protein diets contained 33.2, 22.4, and 11.2% dextrin, respectively. Kitamikado et al. (1964) showed that increased amounts of starch in the diet decreased the digestibility of protein in small (6-g) rainbow trout. Austreng et al. (1977) observed that the use of dietary protein and energy by rainbow trout was poorer when the fish were fed diets with high levels of carbohydrates (up to 38%). Proximate analyses revealed that fish fed high levels of carbohydrate had lower percentages of lipids and energy and higher levels of protein and ash. Although it appears that high levels of metabolizable carbohydrate do affect the digestibility and utilization of protein in some fishes, our proximate analysis data indicate that low protein and high carbohydrate (dextrin) levels in the diets used in this study had no apparent effect on the final composition of tiger muskellunge (Table 3).

Histology

Histological examination of tiger muskellunge showed abundant vacuolization in the livers of fish fed the experimental diets. The vacuoles probably were filled with glycogen in response to dietary dextrin. Liver glycogen increased in common carp (*Cyprinus carpio*), sea bream (*Archosargus rhomboidalis*), and yellowtail (*Seriola lalandei*) when dextrin in the diet was increased from 0 to 40% (Furuichi and Yone 1980). Cowey et al. (1975) found that liver glycogen levels were significantly higher in plaice fed diets containing 20% carbohydrate (dextrin + glucose) than in plaice fed diets without carbohydrate. Carbohydrate in the livers of rainbow trout increased as the level of dietary glucose increased from 0 to 39% (Refstie and Austreng 1981), but no mortality or liver pathology was reported as a consequence of feeding high levels of carbohydrate as glucose or dextrin. Hilton et al. (1981) suggested that increased liver glycogen may result in impaired liver function in rainbow trout. Later, Hilton (1982) suggested that the survival rate of stocked rainbow trout may be reduced in certain circumstances where the fish have elevated liver glycogen resulting from feeding diets high in digestible carbohydrate. Dixon and Hilton (1981) found rainbow trout fed diets with 20% carbohydrate had reduced tolerance to water-borne copper. Although fish in this study did not appear to be negatively affected by the condition of the liver, one cannot ignore the possibility that diets rich in carbohydrates may affect the long-term health and survivability of tiger muskellunge.

Overall, tiger muskellunge held at near optimum growth temperatures made the most efficient use of their diet when it contained 50% protein. Dextrin, used to adjust the energy content of diet, did not appear to affect protein utilization by tiger muskellunge.

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Growth Response of Blue Tilapias to Selected Levels of Dietary Menhaden and Catfish Oils

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Abstract.—Blue tilapia (*Tilapia aurea*) fingerlings were fed a series of nine semipurified diets containing 0, 2.5, 5.0, 7.5 or 10% lipid in the form of menhaden oil or catfish oil over a 10-week experimental period. The study, conducted in flow-through aquaria, demonstrated that best growth and food conversions were obtained from fish fed the 10% menhaden oil diet. Total levels of the linolenic family (ω-3) of fatty acids were highest in the menhaden oil diets (up to 2.5%). In no instance did linolenic acid reach 1.0% of the total diet. That result supports the conclusion that growth reductions observed in tilapia and other fish species fed diets approaching 1% dietary linolenic acid are due to that specific fatty acid, and are not caused by the addictive effect of dietary ω-3 fatty acids.

More rapid growth can be obtained in channel catfish (*Ictalurus punctatus*) from dietary beef tallow or menhaden oil than from vegetable oils when the fish are reared in their optimum temperature range (Stickney and Andrews 1971, 1972). In cool water, averaging 23°C or below, there does not appear to be any growth advantage feeding 6 to 14% beef tallow or menhaden oil (Gatlin and Stickney 1982). The differences in growth response under optimum temperature regimes were once thought due to a depressant effect of high levels of linoleic acid, which is characteristic of vegetable oils. Recent studies, however, have indicated that channel catfish fed dietary levels of 1% or more linolenic acid show depressed growth. Dietary levels of linoleic acid as high as 2% do not depress growth (Stickney et al. 1983). The linolenic acid family (ω-3) of fatty acids, such as eicosahexaenoic acid, are present at relatively high levels in menhaden oil. The level of linolenic acid, however, is low in both fish oil and beef tallow. Practical fish diets containing such vegetable lipids as soybean oil may contain nearly 1% linolenic acid.

Kanazawa et al. (1980) hypothesized that linoleic acid is required by redbelly tilapia (*Tilapia zilli*), and Takeuchi et al. (1983) proposed a 0.5% linoleic acid requirement for *Tilapia nilotica*. Blue tilapia (*Tilapia aurea*) grow well on dietary linoleic acid at levels of up to 2%, but growth is depressed when levels of linolenic acid are at or above 1% (Stickney and McGeachin 1983). A slight decline in blue tilapia growth has also been observed when fish were fed a 10% soybean oil diet as compared with growth on one containing fish oil (Stickney and McGeachin 1985), and the fish did not perform well on beef tallow diets. A subsequent study (Stickney and McGeachin 1984) confirmed the poor performance of blue tilapias on beef tallow diets.

The studies cited above indicate that different responses to dietary lipid quality can be expected from tilapia and channel catfish. Both types of fish grow well in response to diets supplemented with fish oil. The present study was conducted to determine the quantities of menhaden and catfish oil that would produce the best growth in blue tilapia.

Materials

On 1 March 1983, 10 blue tilapias averaging 1.0 g, were stocked into each of 27 20-L-capacity glass aquaria. There were no significant differences ($P < 0.05$) among treatments in initial fish weight. Each aquarium received well water at approximately one exchange every 2 h. Water exited the aquaria from the bottoms via siphon drains. Dissolved oxygen was maintained above 5.0 mg/L by inflow jets and supplemental airstones. The experiment was terminated after 10 weeks on 14 May.

Feed was provided to each aquarium twice daily at 5% of tank biomass per day, with feeding rates adjusted every 2 weeks. The fish were not fed on weighing days.

Nine semipurified diets (Table 1) were offered to fish in triplicate aquaria. In addition to four diets containing menhaden oil and four containing catfish oil, a fat-free diet was prepared. All contained the same levels of casein, vitamins, minerals, and agar. Dextrin and cellulose were used

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TABLE 1.—Percentage composition of experimental diets.

	Dietary lipid percentage				
Ingredient[a]	0.0 (fat free)	2.5	5.0	7.5	10.0
Lipid (menhaden or catfish oil)	0.0	2.5	5.0	7.5	10.0
Dextrin	45.5	39.3	33.1	26.9	20.7
Cellulose	11.2	14.9	18.6	22.3	26.0

[a] Casein was in all diets at 36.2%. Vitamin and mineral levels totalled 6.1% with individual ingredients as in Gatlin and Stickney (1982). Agar was added as a binder at 1%; ethoxyquin antioxidant at 125 mg/kg of diet.

TABLE 2.—Final mean fish weights and food conversion ratios (FCR) for blue tilapias fed experimental diets. Numbers within columns followed by the same letter are not significantly different ($P \leq 0.05$).

Dietary lipid	Final mean weight (g)	FCR (%)
Fat-free	7.8 wv	1.52 zy
2.5% menhaden oil	7.1 v	1.47 zyx
5.0% menhaden oil	9.1 xwv	1.30 yxw
7.5% menhaden oil	11.2 y	1.21 w
10.0% menhaden oil	13.1 z	1.16 w
2.5% catfish oil	7.8 wv	1.70 z
5.0% catfish oil	9.0 xwv	1.27 xw
7.5% catfish oil	9.7 yxw	1.36 yxw
10.0% catfish oil	10.2 yx	1.28 xw

to balance the energy of the fat-free diet with that of the other eight diets. All diets contained 3,600 kcal/kg of energy and 32% protein. Bomb calorimetry was run on the dietary ingredients prior to formulation. The energy level of cellulose was ignored, since that ingredient is not digested by tilapias (Stickney 1976).

Dietary lipids were added after the dry ingredients were thoroughly mixed. Water was added to produce a mixture the consistency of dough. The material was passed through a mechanical meat grinder and the resulting spaghetti-like strands were air-dried and broken into pellets. Two-week supplies of each diet were stored under refrigeration and the rest was frozen. Fatty acid determinations were made by gas chromatography as described by Gatlin and Stickney (1982).

Upon termination of the experiment, mean fish weight and survival were determined for each aquarium. Average weight gains, food conversion ratios (dry weight (g) of food offered per wet weight (g) of fish gain), and survival percentages were calculated and subjected to analysis of variance and Duncan's multiple-range test on the Texas A&M University computer with the use of SAS-79 (Helwig and Council 1979). Significance was accepted at the 0.05 level.

Results and Discussion

No significant differences in final mean weights occurred among fish on diets with 5% lipid (either source) or less. At higher lipid percentages, significant differences occurred (Table 2). Fish on the 10% menhaden oil diet were significantly larger after 10 weeks than those on any other diet. Growth of fish receiving either fish oil diet improved as the level of dietary lipid increased. Levels above 10% dietary lipid were not tested because commercial manufacture of such diets is difficult.

Food conversion ratios generally followed the patterns established for final weights (Table 2). Lowest food conversion ratios were associated with the most rapidly growing fish. Fish on the fat-free diet demonstrated one of the highest food conversion ratios, though the value was not significantly different from fish fed the 2.5 or 5.0% menhaden oil diets or the 2.5 or 7.5% catfish oil diets.

Survival ranged from 93.3 to 100%, with no significant differences among treatments. No disease problems were observed. The mortalities were attributable to fish jumping from their tanks or being injured during weighing.

Fish oils utilized in the study differed considerably with respect to fatty acid composition (Table 3). Linolenic acid was present at 2.3% in menhaden oil and 1.4% in catfish oil. The menhaden oil contained relatively high levels of the ω-3 family fatty acids—linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic. Linolenic family fatty acids accounted for 25.3% of the menhaden oil but only 1.8% of the catfish oil.

Stickney and McGeachin (1983) found that diets supplemented with purified fatty acid esters did not support good growth in blue tilapias when linolenic acid was present at more than 1% of the diet. The species does grow well on diets supplemented with menhaden oil at levels that provide over 1% total ω-3 family fatty acid content. Therefore, growth depression appears to be related to linolenic acid and not higher molecular weight ω-3 fatty acids.

The catfish oil used in this study was obtained from a catfish processing plant and reflected production diets used by catfish farmers. High levels of oleic and linoleic acid in the catfish oil (Table 3) imply that the fish were fed diets high in vegetable oils and perhaps some animal fat.

Redbelly (Kanazawa et al. 1980) and blue tila-

TABLE 3.—Major fatty acids (percentage of total) in menhaden and catfish oil diets.

Fatty acid	Menhaden oil	Catfish oil
Myritic	8.5	2.7
Palmitic	16.8	18.1
Palmitoleic	12.1	6.6
Stearic	6.3	4.5
Oleic	13.2	47.1
Linoleic	2.7	15.6
Linolenic	2.3	1.4
Eicosapentaenoic	13.3	0.2
Docosapentaenoic	2.3	
Docosahexaenoic	7.4	0.2

pias (Stickney and McGeachin 1983) tolerate dietary linoleic acid up to at least 2%. In no instance did dietary linoleic acid exceed 1.6% in the present study. Kanazawa et al. (1980) felt that redbelly tilapias have a dietary requirement for both linoleic and linolenic acid. Their study showed that both fatty acids produce good growth. The positive responses of blue tilapias to the highest levels of both menhaden and catfish oils in the present study may have been due to different fatty acids. Fish on the menhaden oil diets may have responded positively to increasing levels of linolenic acid, while those on catfish oil diets responded to increasing linoleic acid.

In conclusion, this experiment demonstrated that both menhaden oil and catfish oil are excellent sources of dietary lipid for blue tilapia fingerlings. Growth can be substantially improved if either lipid is provided at 7.5–10% of the diet as compared with lower levels. Menhaden oil at 10% of the diet led to best growth and food conversion.

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Effects of Dietary Nonpreferred Aquatic Plants on the Growth of Redbelly Tilapia (*Tilapia zilli*)

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Abstract.—Feeding experiments were designed to compare the growth rate of redbelly tilapia (*Tilapia zilli*) fed four aquatic plants: *Najas marina, Egeria densa, Myriophyllum exalbescens,* and *Chara* sp. Fish ranging in size from 9 to 11 cm total length were divided into five groups, four groups each receiving a plant and one group receiving no food. Redbelly tilapias consumed more *Chara* sp. than any of the other plants. The consumption rate was 79% body weight/d. *Najas marina* was second to *Chara* sp., being consumed at a rate of 67% body weight/d. Fish growth on diets containing *Chara* sp. or *N. marina* did not differ greatly. Fish fed *E. densa* nearly maintained their initial weight although they consumed very little of this plant. Fish fed *M. exalbescens* lost weight, as did the unfed group. The results indicate that control of *Chara* sp. and *N. marina* in small lakes and ponds by redbelly tilapia may be possible.

Aquatic plants often proliferate in shallow waters and interfere with fishing, boating, and other aquatic activities. Chemical control of nuisance plants is costly and can be hazardous to the environment, while mechanical removal is often difficult and costly. These problems have stimulated interest in using herbivorous fish for the biological control of undesirable aquatic plants.

Redbelly tilapia (*Tilapia zilli*), an African cichlid fish, has gained popularity as an agent for weed control, although it does not control all aquatic plants (Hauser et al. 1976), and shows preferences when feeding choices are offered (Fitzpatrick 1978). Although redbelly tilapias eradicated *Chara* sp. and *Najas marina* from a pond in the southeastern United States, *N. marina* was consumed only after the *Chara* sp. was eliminated (Rickel 1975). Fitzpatrick (1978) found that the fish prefer soft plants to coarse ones. *Chara* sp. and *Zanichellia palustris,* both soft textured plants, were preferred to *N. marina* and *Myriophyllum exalbescens.*

Redbelly tilapias will consume nonpreferred plants, if nothing else is available (Legner 1977). The extent to which the fish consume nonpreferred plants has not been determined, nor has growth of the fish eating nonpreferred plants been measured. Redbelly tilapias gained weight in aquaria containing a combination of *Najas guadalupensis, Potamogeton pectinatus, Chara* sp., and *Myriophyllum spicatum.* The fish lost weight when *M. spicatum* was fed alone (Hauser and Legner 1974). They showed a diet shift with size increase, and fish over 9.0 cm length were better able to eat macrophytes than smaller size fish (Fitzpatrick 1978; Legner 1978).

The objective of this study was to compare growth of redbelly tilapias fed different species of plant, the preferred *Chara* sp. and the nonpreferred *N. marina, M. exalbescens,* and *E. densa.*

Methods

Growth rates of redbelly tilapias fed four aquatic plants—*Chara* sp., *E. densa, N. marina, and M. exalbescens*—were measured. Two controls were used concurrently: one group was fed *Chara* sp., which is known to be readily consumed, and the other group received no food.

Redbelly tilapias were obtained from stock maintained by the Arizona Cooperative Fishery Research Unit, University of Arizona, Tucson. Fish selected were 9–11 cm total length (TL), the size range in which they are predominantly herbivorous. Each of five 300-mL tanks were divided into 10 compartments and stocked with one randomly selected fish per compartment, providing ten fish for each treatment. Fish were acclimated to experimental tanks for 48 h prior to weighing, then weighed in water to the nearest 0.1 g. Water temperatures ranged from 25 to 30°C and dissolved oxygen was maintained by aeration between 6.8 and 8.8 mg/L.

Plants were obtained fresh from a nearby lake

TABLE 1.—Food consumption and growth of redbelly tilapia fed four different plant species. Controls are fish which were not fed.

Variable	*Chara* sp.	*N. marina*	*E. densa*	*M. exalbescens*	Not-fed
Mean weight consumed per fish per day (g)	14.8	11.6	4.9	2.9	
Food consumption (% body weight/d)	79	67	24	16	
Mean fish weight (g)±SE					
Initial	17.86±0.72	16.73±0.27	20.54±0.70	19.58±0.77	18.97±0.60
Final	19.44±0.82	17.93±0.51	20.39±0.77	15.61±0.51	15.27±0.52
Mean percent change in body weight±SE[a]	+8.80±2.11 z	+7.20±1.99 z	−0.72±0.96 y	−20.30±1.39 x	−19.51±1.32 x
Slope coefficient[b]	+0.33 w	+0.24 v	−0.02 v	−1.0 w	−0.88 w

[a] Means followed by a common letter are not different ($P > 0.01$), analysis of variance.

[b] Values followed by the letter w are different from zero; those followed by the letter v are not ($P > 0.05$), *t*-test.

and damp dried by vigorous shaking and applying a paper towel. Plants were weighed before being placed in compartments with fish; they were added or removed every other day, so that fresh plant material was always available as food for the fish. When plants were removed, damp-dry weights were recorded and subtracted from the weight introduced to give the amount consumed. Fish were weighed at 14-d intervals. The experiment was terminated after 56 d. Results were compared using standard statistical tests. Fish behavior and mode of eating each plant species were studied in glass aquaria located in a dark room, in which light was adjusted using a time clock to duplicate day and night periods. Small holes were made in the dark room wall so fish could be observed without disturbance.

Results

Food consumption and growth of redbelly tilapias are shown in Table 1. When growth rates (slope coefficients) were compared to zero (zero slope equals no growth), the only statistically significant growth occurred in the group of fish fed *Chara* sp. The fish gained weight on *N. marina*, but to a lesser degree than on *Chara* sp. Fish fed *E. densa* maintained approximately their original weight, while those fed *M. exalbescens* lost weight.

The growth of redbelly tilapias fed *Chara* sp. was 0.03 g/d. The conversion ratio, using the amount of plant consumed and the average growth of fish, was only 500:1. Average growth of fish fed *N. marina* was 0.02 g/d; the conversion ratio was 580:1. Loss of weight by fish fed *E. densa* was negligible. Fish fed *M. exalbescens* markedly lost weight, which was similar to the group of fish fed nothing ($P \leq 0.01$).

Observations of redbelly tilapias feeding on *Chara* sp. showed that the fish approached the plant, grasped the branch with their jaws, gradually filled their mouths, and then separated the branch from the main plant. They appeared to grind the plant with the pharyngeal teeth and swallow the whole bite. Fish feeding on *N. marina* began feeding on the adventitious roots, if they were present, and then took the lower parts of the stems where leaves are less dense. They left the terminal bushy twigs untouched. The fish fed sparingly on *E. densa*. They took leaves and slender stems. They were rarely observed eating *M. exalbescens*. On many occasions the fish were seen to bite the plant, but then spit the plant material out.

Discussion

Growth of redbelly tilapias fed four aquatic plants varied, depending on the plant species. This variability was attributed to differences in consumption rates. An evaluation of these results provides a basis for predicting the potential of redbelly tilapia to control aquatic plants.

Rickel (1975) stated that plant preference is mainly determined by coarseness. This does not seem to be true for *Chara* sp. which was readily consumed by redbelly tilapias in this study. *Chara* sp. could be considered a coarse plant due to encrustation by calcium carbonate (Boyd 1968).

The high consumption of *N. marina* by redbelly tilapias recorded here indicates that the fish will eat this species and will increase in weight when no other food is offered. Although growth using *N. marina* was not statistically different from zero, it was comparable to *Chara* sp., with 7.20% and 8.80% increase in body weight, respectively. Coarseness of *N. marina* appears to have some influence on its consumption by the fish. This plant has characteristically sharp-toothed leaf margins. Fish avoided the terminal bushy twigs on which the leaves are crowded while taking stems and lower leaves, probably because the spines are less numerous.

Redbelly tilapias fed *E. densa* consumed little, and their lack of growth could be attributed to the small amount consumed. Fish avoided the bulky stems and fed on leaves and soft slender stems which were easy to grasp and separate.

The fish fed *M. exalbescens* lost weight and consumed very little. This plant has a high cellulose content, is difficult for fish to bite, and is coarse due to encrustation by calcium carbonate on the external surfaces (Boyd 1968). These characteristics make *M. exalbescens* high in indigestible materials and apparently unpalatable.

Lack of growth suggests possible nutritional deficiencies. Boyd (1968) determined the proximate nutritional analysis of *E. densa*, *M. spicatum* (which resembles *M. exalbescens* very closely), *M. brasiliense*, *Chara* sp., and *N. guadalupensis*. *Egeria densa* has a high crude protein content (20.5% dry weight) compared to the analysis of other plants. High protein content may explain why the fish maintained their body weight despite their low consumption rates of *E. densa*. Leaves are generally high in protein (Boyd and Goodyear 1971), and it is likely that fish fed selectively on *E. densa* leaves.

Rejection of *M. exalbescens* by the fish and the subsequent loss of weight may be caused not only by a lack of palatability but also by a lack of some essential nutrients. When Nelson and Palmer (1938) provided the right supplements of proteins, fat, and vitamins, *M. spicatum* was taken readily by rats; this could also be the case with redbelly tilapia.

The growth of fish in the group fed *Chara* sp. was not outstanding. Fish consumed this plant at a rate of 79% of their body weight per day. When the actual gain in weight was considered, it became evident that *Chara* sp. had a poor weight conversion ratio, and growth achieved was considered inadequate to produce a sport or commercial fishery. When redbelly tilapias were offered a choice of *Chara* sp. and tubifex worms, they ate about 50% of each type of food; worms were offered by number and plants by weight (Fitzpatrick 1978).

Apparently redbelly tilapia is not as herbivorous as it was thought to be at this age, and needs animal nutrients. The fish adapt readily to different diets and they consume a variety of foods. Stomach analyses of wild fish indicated that food varied from algae, macrophytes, and detritus to zooplankton, benthos, and pleustonic insects (Spataru 1978).

Ponds and lakes usually have more than one species of plant. From the results presented, it seems possible that redbelly tilapia may control *Chara* sp. and *N. marina* in ponds, but with a low growth rate unless animal food supplements are used. *E. densa* and *M. exalbescens* would probably need alternative control methods.

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Environmental Factors Affecting Flavor of Channel Catfish from Production Ponds

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Abstract.—Environmental variables and the flavor of channel catfish (*Ictalurus punctatus*) were monitored at 3–4-week intervals during 1982 and 1983 in 11 ponds at Browns, Alabama, and during 1983 in five production ponds near Auburn, Alabama. Off-flavor in fish flesh was most severe in late summer when algal and actinomycete activity in ponds was greatest in response to high water temperatures and large daily applications of fish feed. However, some fish from all ponds were not off-flavor. For example, in August 1982, flavor scores for fish from 11 ponds at Browns ranged from 1.6 to 9.5 (a score above 9 indicates no off-flavor). The earthy–musty flavor of geosmin was the most common and intense off-flavor in fish. Regression analyses between environmental variables and flavor scores failed to yield strong or consistent correlations. Blue-green algae and actinomycetes, which are known to produce geosmin and other odorous compounds, were abundant in ponds with off-flavor fish; however, these microorganisms also were abundant in ponds where fish were not off-flavor. Off-flavor was equally common in fish from ponds with acidic muds and low alkalinity waters and from ponds with alkaline muds and high alkalinity waters, but the lowest flavor scores were recorded in the more alkaline environments.

Off-flavor in pond-reared fish has been identified by the Catfish Farmers of America trade organization as the most serious problem in the catfish industry (Lovell 1983a). Processors routinely conduct taste tests of channel catfish (*Ictalurus punctatus*) and normally refuse to purchase fish that are off-flavor. As a result, farmers must hold these fish in ponds until flavor improves. Inability to sell fish according to a predictable schedule is a serious economic disadvantage to farmers.

Off-flavor in fish is caused by the absorption of objectionable-flavor compounds by the fish from the environment (Thaysen 1936; Lovell and Sackey 1973; Persson 1979). Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB) cause the earthy–musty flavor in fish (Persson 1980; Yurkowski and Tabachek 1980). Lovell (1983b) classified several new off-flavors—sewage, stale, rancid, metallic, moldy, petroleum, and weedy—in pond-reared channel catfish. Specific compounds responsible for these off-flavors were not identified.

Geosmin and MIB are synthesized by blue-green algae and actinomycetes and are excreted into the environment (Safferman et al. 1967; Henley 1970). A number of other compounds with earthy–musty odors also are synthesized by actinomycetes (Gerber 1977). Lovell and Sackey (1973) showed that channel catfish acquired off-flavor when held in water containing the blue-green algae *Symploca muscorum* and *Oscillatoria tenuis* or in algae-free filtrates of water that had contained the two algae. Channel catfish ponds often have high densities of blue-green algae (Boyd 1979). Because earthy–musty flavor in fish was associated with blue-green algae (Lovell and Sackey 1973; Tabachek and Yurkowski 1976), it has been assumed that off-flavor compounds tainting channel catfish originate primarily from blue-green algae (Lovell 1983a, 1983b). However, the role of other microorganisms cannot be dismissed. Actinomycetes no doubt are abundant in catfish ponds, and many species of green algae and diatoms have been associated with odorous conditions in water-supply reservoirs (EPA 1977).

Little is known about factors influencing the occurrence of off-flavor in pond-reared channel catfish. A survey of catfish farms in Alabama and Mississippi disclosed the following: fish from 50% or more ponds could be unmarketable because of off-flavor during summer or early fall; off-flavor occurred more frequently in areas with heavy, alkaline soils than in areas with sandy, acidic soils; off-flavor fish may be found in old or new ponds; off-flavor may occur at both low and high feeding rates (Lovell 1979). Brown and Boyd (1982) collected samples of fish from 23 commercial catfish ponds in Alabama during September. Fish from all ponds had detectable off-flavor, and the off-flavor was more intense at high feeding rates.

In an attempt to define environmental conditions associated with off-flavor in channel catfish, fish were collected for flavor testing over two growing seasons from ponds on a commercial fish farm

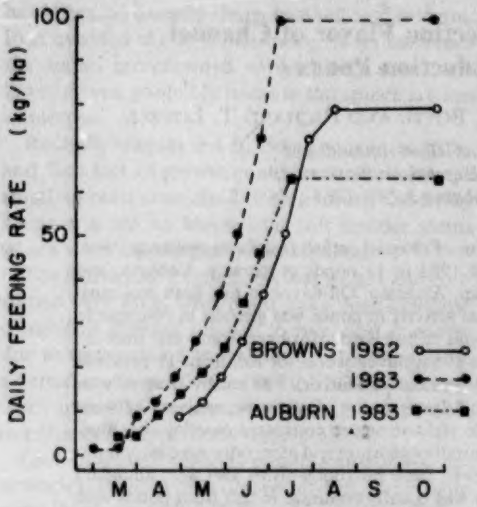

FIGURE 1.—Daily feeding rates for channel catfish in ponds at Browns, Alabama, and ponds near Auburn, Alabama, between March and October.

and over a single growing season from ponds on a research station.

Methods

Eleven catfish ponds (4 to 12 hectares) were located on a fish farm at Browns in Dallas County, Alabama. Eight of these ponds were filled and their water levels maintained by runoff from pastures or woodland; they were drained annually for fish harvest. The other three ponds were supplied with well water. Fish were harvested by seining, and the ponds were last drained in 1980. Ponds at Browns were constructed on heavy, slightly alkaline soils of the Black Belt Prairie. Five ponds (0.8 to 9.0 hectares) were located on the Auburn University Fisheries Research Unit near Auburn in Lee County, Alabama. Runoff from wooded watersheds supplied water to these ponds, and they had been drained annually for the past decade to permit fish harvest. The ponds were constructed on light, acidic soils of the Piedmont Plateau.

Ponds at Browns were stocked at 15,000 and 16,000 channel catfish (36 kg/1,000 fish) per hectare in 1982 and 1983, respectively. Ponds near Auburn were stocked at 14,000 channel catfish (34 kg/1,000 fish) per hectare in 1983. Fish in all ponds were fed a floating, pelleted feed that contained 32% crude protein. Daily feeding rates are presented in Figure 1; maximum daily rates were 88 kg/hectare in 1982 and 100 kg/hectare in 1983 at Browns and 64 kg/hectare in 1983 at Auburn.

Pond sampling at Browns was conducted at approximately 3-week intervals between May and October 1982. In 1983, ponds at Browns and those near Auburn were sampled on a monthly basis.

Water samples for chemical analysis and phytoplankton enumeration were collected at one location in each pond by holding open 1-L polyethylene bottles 10 cm below the surface. Water samples for chemical analysis were stored on ice and analyzed within 12 h after collection. Phytoplankton samples were preserved with Merthiolate (APHA et al. 1980). Water samples for actinomycete enumeration were obtained by filling sterile 500-mL glass bottles as described above. Mud samples for actinomycetes were obtained from five places in each pond with a 7.6-cm-diameter core sampler. Mud was transferred to sterile 500-mL glass jars and stored on ice. Two catfish were caught from each pond by hook and line pithed, and placed on ice. At the laboratory, fish were deheaded, gutted, skinned, and filleted. They were then frozen until used in flavor tests.

Surface water temperatures were measured each sampling date. All water samples were analyzed for chemical oxygen demand and chlorophyll *a* and, on three dates each year, determinations of total alkalinity and total hardness were made. Analyses followed methods outlined by APHA et al. (1980). Phytoplankters were concentrated by sand filtration (Boyd 1979); they were enumerated and identified to genus in a Sedgewick–Rafter counting chamber under 150× magnification. Enumeration consisted of recording the number of individual phytoplankters (single cells, colonies, or filaments) of each genus. Samples for actinomycete enumeration were diluted and plated by a double-layer agar technique involving a 10-mL base layer of agar in a 90-mm-diameter Petri dish overlayed with 5 mL of molten agar containing the diluted sample. The growth media was starch–casein agar (APHA et al. 1980) with 1 g/L of cycloheximide for fungal control. Rose bengal at 0.1 g/L was added to the upper layer to enhance the visibility of actively growing actinomycete colonies. Each sample was plated in triplicate. Petri dishes were inverted and incubated for 5–7 d at 28°C in the dark. Actinomycetes were distinguished from other bacteria and fungi by their tough, leathery colonies that grew into the media, by the presence of aerial mycelia, and by their red color from absorbing rose bengal. Only those dilutions which resulted in 30 to 300 colonies per plate were considered reliable.

A three-member flavor panel was able to detect

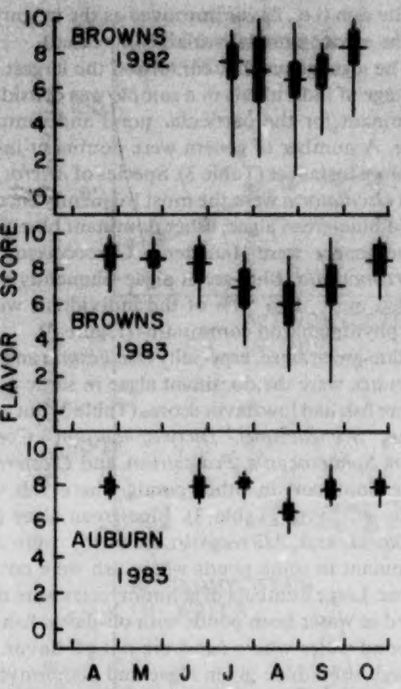

FIGURE 2.—Summary of flavor scores (0 = extreme off-flavor; 10 = no off-flavor) for channel catfish collected between April and October from 11 ponds at Browns, Alabama, and from five ponds near Auburn, Alabama. Horizontal lines, rectangles, and vertical lines represent means, standard deviations, and ranges, respectively.

as little as 0.5 μg/L of geosmin in water in a threshold screening test conducted according to Amerine et al. (1965). Procedures for training the panel to recognize the different types of off-flavor were described by Lovell (1938b). For flavor tests, fillets were wrapped in aluminum foil and cooked for 15–20 min at 250°C in a conventional electric oven. The panel evaluated the degree of off-flavor using the scoring scale of Lovell and Sackey (1973): no off-flavor (9–10); slight off-flavor (7–8.9); distinct off-flavor (5–6.9); intense off-flavor (3–4.9); extreme off-flavor (0–2.9). The type of off-flavor was recorded as geosmin (earthy–musty), stale, detritus, chemical, sewage, plant, or cardboard. Between five and 10 fish samples were evaluated at one time. The origin of the samples was not disclosed to panelists until scoring was completed. Most samples were used in taste tests within 4 d after collection.

TABLE 1.—Percentage distribution of flavor scores in samples of channel catfish collected during April through October from 11 ponds at Browns, Alabama, and five ponds near Auburn, Alabama.

Score	Degree of off-flavor	Browns 1982	Browns 1983	Auburn 1983
9–10	None	29.4	31.5	12.0
7–8.9	Slight	31.4	48.0	68.0
5–6.9	Distinct	21.6	12.3	20.0
3–4.9	Intense	11.8	5.5	0.0
0–2.9	Extreme	5.9	2.7	0.0

Test for significance among means for flavor scores and for environmental data were made with t-tests (two means) or with Duncan's new multiple-range test (three or more means).

Results and Discussion

Flavor scores differed greatly among ponds on individual dates (Figure 2), but mean flavor scores obtained in August were lower ($P < 0.01$) than those for other months. The lowest flavor scores for fish from individual ponds also were recorded in August, but scores below 5.0 were obtained from July to October at Browns. Mean flavor scores did not differ ($P > 0.05$) between Browns and Auburn; however, scores below 5.0 were not recorded for fish from ponds at Auburn. Considering all ponds and sampling dates, most ponds contained off-flavor fish, but fewer than 20% of the scores were 5.0 or less (Table 1). Several types of off-flavor were found and the geosmin and sewage

TABLE 2.—Percentage occurrence of fish flavors and average flavor scores for channel catfish collected during April through October from 11 ponds at Browns, Alabama, and five ponds near Auburn, Alabama.

Flavor	Browns 1982	Browns 1983	Auburn 1983	Average flavor score[a] for all samples
None[b]	31.4	40.5	12.0	9.2
Stale	2.0	5.4	32.0	8.2
Detritus	2.0	12.2	4.0	7.7
Sewage	19.6	12.2	12.0	7.5
Cardboard	7.8	1.3	0.0	7.5
Chemical	2.0	2.7	0.0	7.3
Plant	0.0	1.3	8.0	7.3
Geosmin	35.2	24.3	32.0	5.5

[a] No off-flavor (9–10); slight off-flavor (7–8.9); distinct off-flavor (5–6.9); intense off-flavor (3–4.9); extreme off-flavor (0–2.9).

[b] Does not agree with Table 1 percentage of fish with no off-flavor (Browns) because the type of flavor was not identified for a few samples.

tastes were encountered most frequently (Table 2). The intensity of the geosmin off-flavor was greater than that of the other types of off-flavor.

Total alkalinity of pond water ranged from 35 to 150 mg/L as $CaCO_3$ (mean = 75 mg/L) at Browns and from 8 to 15 mg/L (mean = 12 mg/L) at Auburn. Total hardness concentrations were approximately equal to total alkalinity at both locations.

Other environmental data are summarized in Figure 3. On individual dates, variation among ponds in chemical oxygen demand, chlorophyll *a*, actinomycetes, and phytoplankton was great and differences in these variables between years at Browns and between Browns and Auburn were not significant ($P > 0.05$). Differences in magnitude of environmental variables among dates were not tested for significance, but the largest mean values often were noted in August. Flavor scores also averaged lowest at this time (Figure 2).

Linear and step-wise multiple regression analyses were conducted for environmental variables (X) and off-flavor scores (Y). Data were separated for regression analysis into the following categories: Browns 1982, Browns 1983, Auburn 1983, and all ponds and dates. Coefficients of determination (r^2) for linear regressions between all environmental variables, except actinomycetes in the mud and flavor scores, were significant ($P < 0.05$). However, with one exception, r^2 values were below -0.50. In 1982 at Browns, the coefficient of determination between the abundance of blue-green algae and flavor scores was -0.91. The r^2 values did not improve appreciably in step-wise regressions. Brown and Boyd (1982) found that flavor score decreased with maximum daily feeding rate in 23 channel catfish ponds where peak daily feed applications ranged from 19 to 132 kg/hectare. In the present study, there was little variation in maximum daily feeding rates and, among ponds, there was no relationship between feeding rate and flavor score.

Regression analyses were also conducted on environmental data and flavor scores for individual ponds. Coefficients of determination of -0.70 to -0.95 were obtained in some ponds for the regressions between individual variables (feeding rate, chlorophyll *a*, phytoplankton density, abundance of blue-green algae, chemical oxygen demand, abundance of actinomycetes in the water, and water temperature) or between various combinations of these variables and flavor scores. However, in other ponds, coefficients of determination were not significant, or they were significant but with opposite sign (i.e., flavor improved as the magnitude of the environmental variable increased).

The algal genus that comprised the largest percentage of individuals in a sample was considered dominant for the particular pond and sampling date. A number of genera were dominant in one or more instances (Table 3). Species of *Microcystis* and *Oscillatoria* were the most frequently encountered blue-green algae; other dominant blue-green algae genera were *Anabaena*, *Chroococcus*, and *Merismopedia*. Blue-green algae frequently comprised more than 50% of the individuals within the phytoplankton community (Figure 3).

Blue-green algae, especially *Oscillatoria* and *Microcystis*, were the dominant algae in some ponds where fish had low flavor scores (Table 3) but green algae, *Scenedesmus*, *Dictyosphaerium*, *Coelastrum*, *Sphaerocystis*, *Pediastrum*, and *Closterium*, were dominant in other ponds where fish were badly off-flavor (Table 3). Blue-green algae (*Oscillatoria* and *Microcystis* included) were also dominant in some ponds where fish were not off-flavor. Large numbers of actinomycetes were measured in water from ponds with off-flavor fish and in pond water where fish were not off-flavor. Although some blue-green algae and actinomycetes are known to produce odorous substances, findings presented above do not demonstrate a simple or clear-cut relationship between the abundance of these organisms and off-flavor in channel catfish. Furthermore, the results do not rule out the possibility that other forms of algae may contribute to off-flavor.

Results imply that off-flavor is most severe in late summer when algal and microbial activity in ponds is high in response to large daily applications of feed. However, there was considerable variation among ponds with respect to off-flavor scores. For example, in August 1982 all ponds at Browns received daily feed applications of 88 kg/hectare, they had dense algal communities, and actinomycetes were abundant. Nevertheless, flavor scores ranged from 1.6 to 9.5.

Earlier studies suggested that off-flavor in channel catfish was more common in ponds on heavy, alkaline soils than in ponds on sandy, acidic soils. In the present study, off-flavor was common in ponds near Auburn that were constructed on light, acidic soils and had low concentrations of total alkalinity and total hardness. However, fish from ponds at Auburn did not have flavor scores as low as some that were recorded from ponds at Browns, where soils were heavy and alkalinity and hardness were high. The off-flavor problem is obviously

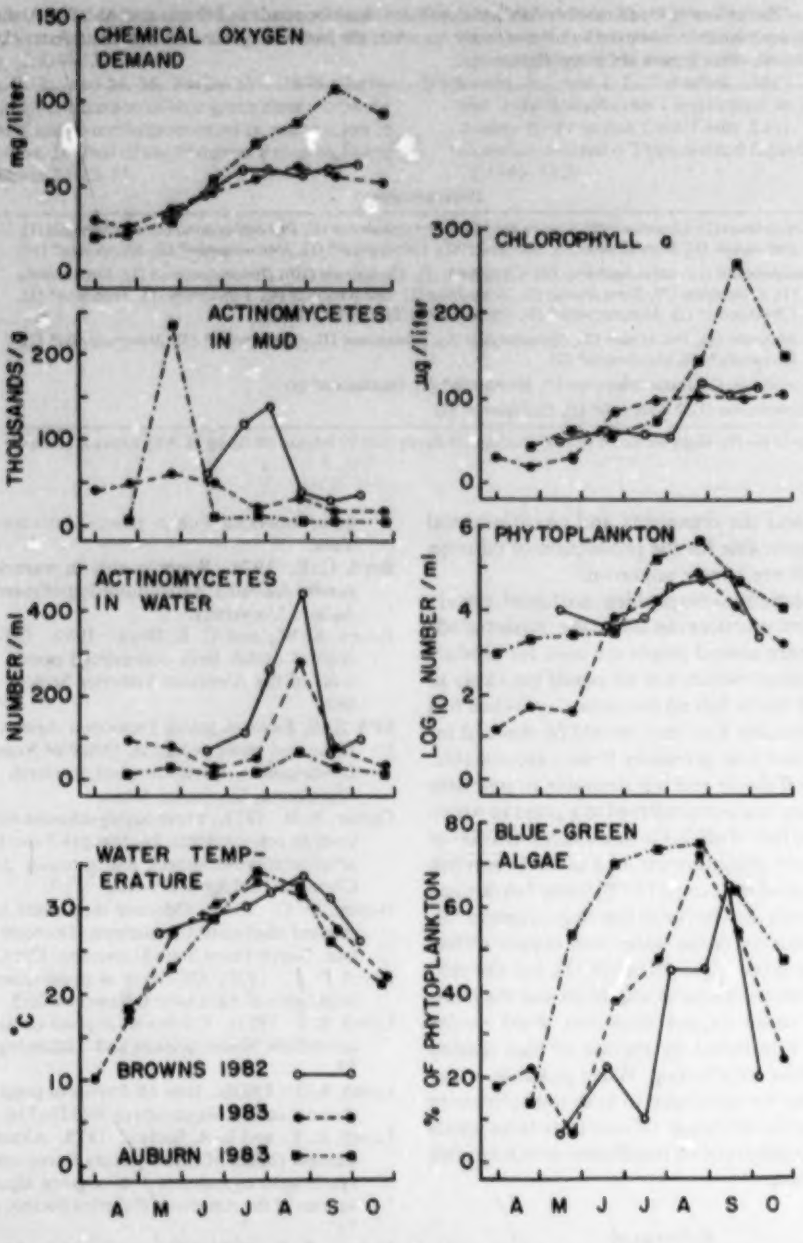

FIGURE 3.—Summary of limnological variables between April and October in 11 channel catfish ponds at Browns, Alabama, and five channel catfish ponds near Auburn, Alabama.

TABLE 3.—Flavor scores for channel catfish and dominant algae for ponds at Browns and Auburn, Alabama. The numbers in parentheses indicate the number of instances where the particular genus was dominant. Asterisks indicate blue-green algae; other genera are green algae.

Score[a]	Dominant algae	Dominance by blue-green algae (%)
9–10	*Actinastrum* (2), *Closterium* (8), *Coelastrum* (5), *Dictyosphaerium* (1), *Dimorphococcus* (1), *Kirchneriella* (1), *Pediastrum* (4), *Scenedesmus* (1), *Anabaena** (2), *Chroococcus** (1), *Merismopedia** (2), *Microcystis** (10)	42.5
7–8.9	*Actidesmium* (1), *Ankistrodesmus* (2), *Closterium* (2), *Coelastrum* (10), *Dimorphococcus* (1), *Kirchneriella* (1), *Pediastrum* (7), *Scenedesmus* (5), *Schroderia* (1), *Sphaerocystis* (4), *Tetrastrum* (1), *Anabaena** (1), *Chroococcus** (2), *Merismopedia** (3), *Microcystis** (22), *Oscillatoria** (6)	56.2
5–6.9	*Coelastrum* (4), *Pediastrum* (2), *Sphaerocystis* (1), *Starastrum* (1), *Chroococcus** (3), *Merismopedia** (2), *Microcystis** (9), *Oscillatoria** (3)	68
3–5.9	*Coelastrum* (2), *Dictyosphaerium* (1), *Microcystis** (2), *Oscillatoria** (4)	66.7
0–2.9	*Scenedesmus* (1), *Microcystis** (2), *Oscillatoria** (2)	80

[a] No off-flavor (9–10); slight off-flavor (7–8.9); distinct off-flavor (5–6.9); intense off-flavor (3–4.9); extreme off-flavor (0–2.9).

complex, and the organisms and environmental factors responsible for the production of odorous compounds are largely unknown.

Until solutions to the problem are found, certain management practices can lessen the impact of off-flavor. Where several ponds are used for production of channel catfish, not all ponds are likely to contain off-flavor fish on the same day. When fish reach marketable size, they should be checked for off-flavor and sold promptly if they are suitable. If fish are off-flavor and it is desirable to sell them at once, they can be transferred to a pond in which the water is free of off-flavor substances. Off-flavor generally will disappear within 5 to 10 d from fish held in clean water (Lovell 1979). Some fish farmers have reported that flavor in fish may improve following treatment of the water with copper sulfate to kill blue-green algae (Boyd 1979), but the reliability of this technique is unproven and the treatment can cause oxygen depletion. Feed wastes should be minimized by the use of high quality feeds without overfeeding. When possible, water in ponds can be exchanged to flush out substances responsible for off-flavor. Of course, in large ponds the water supply is often insufficient to accomplish rapid flushing.

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Effects of Annual Draining on the Limnology of Sunfish Ponds

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Abstract.—Average concentrations of total phosphorus and chlorophyll *a* increased annually over a 3-year period in fertilized sunfish ponds that were not drained. Average concentrations of these variables remained approximately the same each year in ponds that were drained annually. Greater phytoplankton production in the undrained ponds, as evident from larger chlorophyll-*a* concentrations, was attributed to increased availability of phosphorus from sediments. Because phosphorus accumulates in sediments of undrained ponds, fertilization rates likely can be reduced in many ponds with a history of fertilization.

Ponds used for research on pond fertilization usually are drained each year to permit harvest of all fish and then refilled (Dobbins and Boyd 1976; Boyd 1981). When fish ponds are drained, waters containing high concentrations of nutrients are discharged and replaced with waters of lower fertility. During draining and seining operations, nutrient-enriched sediments in ponds are brought into suspension and discharged (Boyd 1978). This annual nutrient loss during draining of experimental ponds may eliminate any residual effect of pond fertilization. Swingle et al. (1965) were unable to demonstrate a residual effect of phosphate fertilization in ponds that had been used in fertilization experiments and drained annually for 15 to 20 years. However, Eren et al. (1977) found that muds in undrained, fertilized ponds in Israel gradually decreased in ability to remove phosphorus from water, they suggested that phosphorus application rates likely could be lowered after several years of fertilization. Therefore, the present research was conducted to compare limnological conditions in annually drained and undrained sunfish ponds.

Methods

Eight earthen ponds (0.04–0.07 hectare) used in this study are located at the Auburn University Fisheries Research Unit, Auburn, Alabama. Pond depths averaged 1.0 m and ranged from 0.3 m at the edges to 1.5 m at the standing drain pipes. Water levels were maintained by additions of soft (total hardness and total alkalinity of 8–12 mg/L as $CaCO_3$), infertile (total ammonia nitrogen, 0.01–0.05 mg/L; nitrate-nitrogen, 0.01–0.03 mg/L; total phosphorus, 0.01–0.02 mg/L) water. No appreciable outflow occurred.

The study extended over three growing seasons (1981–1983). Ponds were limed each year in December with 2,240 kg/hectare of agricultural limestone. In January 1981, all ponds were stocked with 5,400 bluegills (*Lepomis macrochirus*) per hectare. Bluegills were 3 to 8 cm in total length. Each year the same four ponds were drained and fish were harvested in October; these ponds were refilled within 1 or 2 weeks and bluegills were stocked again in January of the following year. The other four ponds were not drained nor were fish removed from them until October 1983. Fertilization was initiated in late February each year with diammonium phosphate ($N–P_2O_5–K_2O$: 18–46–0) at 9 kg of P_2O_5/hectare application. Three applications were made at 2-week intervals, followed by monthly applications until early October. Fertilizer was dissolved in water and splashed over pond surfaces.

Limnological variables were determined periodically between March and October. Water samples were collected with a 90-cm column sampler (Boyd 1979), poured in plastic bottles, and analyzed at once. Chlorophyll *a* (acetone extraction and spectroscopy) was measured every 2 weeks, and total phosphorus (sulfuric acid–persulfate digestion; ascorbic acid method), filterable orthophosphate (ascorbic acid method), nitrate-nitrogen (cadmium reduction method), and total ammonia nitrogen (phenate method) were measured every 2 months the first year. During the second and third years, chlorophyll *a* was measured every 2 weeks, while total phosphorus, filterable orthophosphate, nitrate-nitrogen, and total ammonia nitrogen were measured every 4 weeks. All procedures followed protocol suggested by APHA et al. (1980).

Sediment samples were collected from the upper 5-cm layer each spring and fall while ponds were full, and after final draining in 1983. Five samples

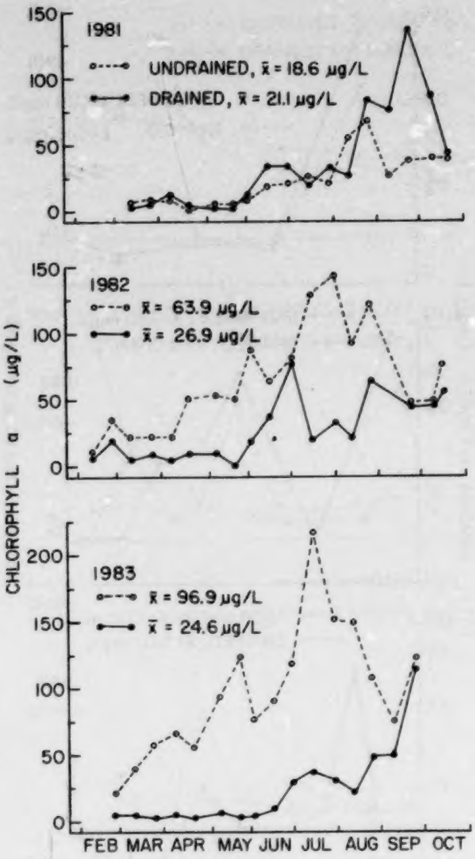

FIGURE 1.—Chlorophyll-*a* concentrations in fertilized sunfish ponds, 1981–1983 ($\bar{x}$ = mean).

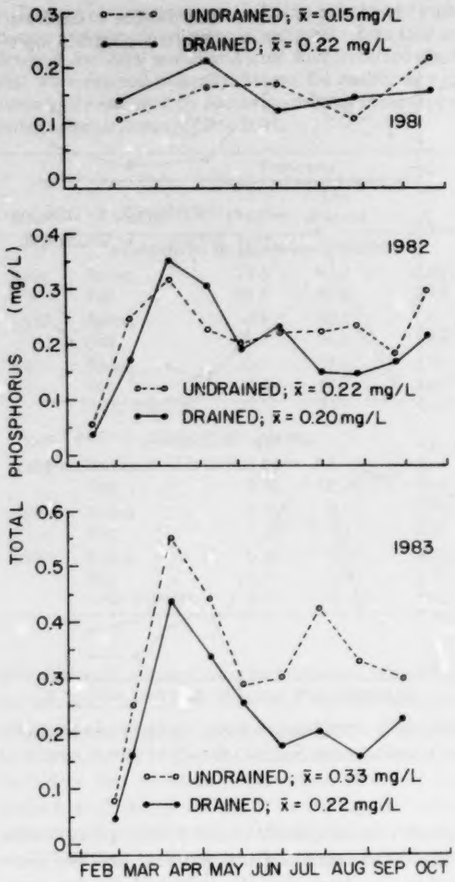

FIGURE 2.—Total phosphorus concentrations in fertilized sunfish ponds, 1981–1983 ($\bar{x}$ = mean).

were taken from pond bottoms (shallow and deep ends) and composited. Each sample was air dried, pulverized, and passed through a 0.85-mm screen. Organic nitrogen was determined according to Jackson (1958). Dilute acid-extractable phosphorus was determined by the Auburn University Soil Testing Laboratory.

Results and Discussion

Concentrations of chlorophyll *a*, total phosphorus, filterable orthophosphate, total ammonia nitrogen, and nitrate-nitrogen at intervals during each growing season and annual means for these variables in both treatments are presented in Figures 1–5. Statistical analysis with *t*-tests (Steel and Torrie 1960) revealed no significant differences between treatments in these variables ($P > 0.05$) on individual sampling dates or for annual means during the first year. On several dates during the second year, concentrations of chlorophyll *a* and total phosphorus were significantly higher ($P < 0.05$) in undrained ponds than in drained ponds, and the annual mean for chlorophyll-*a* concentration was greater for the undrained ponds ($P < 0.01$). In the third year, concentrations of chlorophyll *a* and total phosphorus were usually greater on individual sampling dates in undrained ponds; seasonal means for these two variables were also higher for undrained ponds ($P < 0.01$). Variability in nitrate-nitrogen and total ammonia nitrogen concentrations among ponds within a treatment was large; no significant differences in the concentrations of these two nutrients were determined during the second and third years.

Although nitrogen fertilization is sometimes

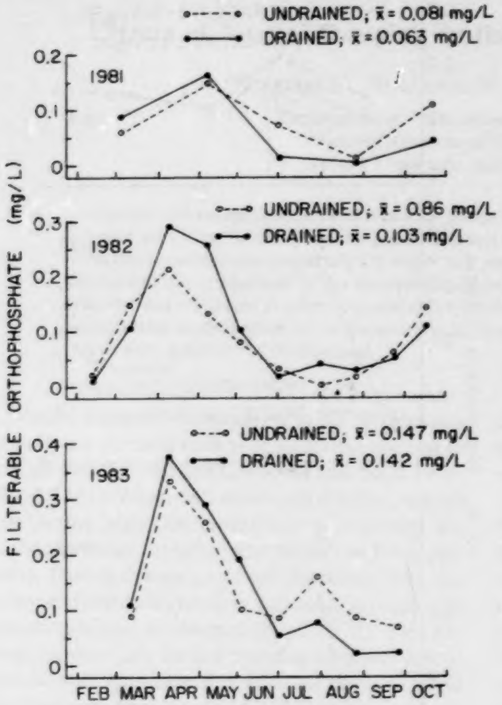

FIGURE 3.—Filterable orthophosphate concentrations in fertilized sunfish ponds, 1981–1983 (x̄ = mean).

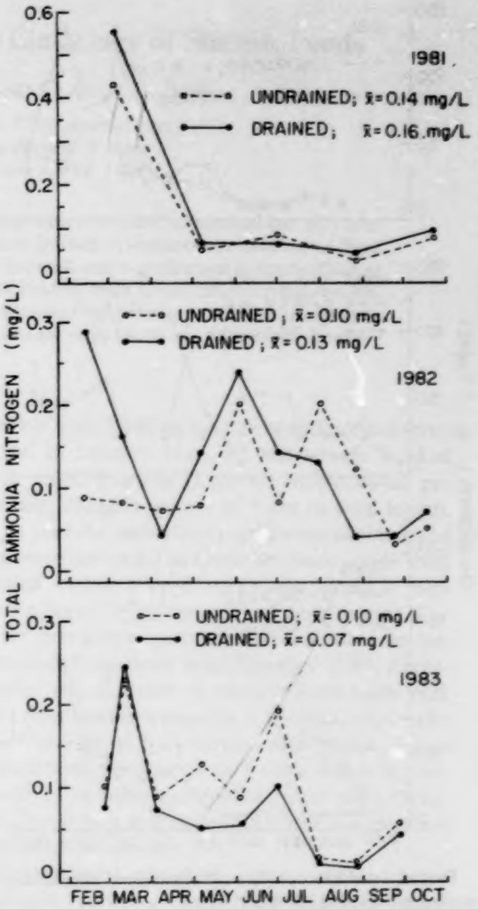

FIGURE 4.—Total ammonia nitrogen concentrations in fertilized sunfish ponds, 1981–1983 (x̄ = mean).

necessary in fish ponds (Boyd 1982), phosphorus is usually the most critical nutrient limiting production. Furthermore, the potential for fish production increases with increasing phytoplankton abundance and chlorophyll-*a* concentrations (Boyd 1982). Annual means for concentrations of chlorophyll *a* and total phosphorus in sunfish ponds at Auburn University that were drained annually in fertilization experiments have not exceeded 40 μg/L and 0.25 mg/L, respectively (Boyd 1982). In the present research, the annual mean for chlorophyll *a* exceeded 40 μg/L during the second and third years; the annual mean for total phosphorus exceeded 0.25 mg/L during the third year.

While acid-extractable phosphorus was significantly greater ($P < 0.05$) in sediments of the undrained treatment in the fall of 1983 (Table 1), no other differences in acid-extractable phosphorus or organic nitrogen in sediments were significant ($P > 0.05$). The accumulation of phosphorus in sediment of fertilized ponds is in agreement with findings of Eren et al. (1977) for fish ponds in Israel. The greater average chlorophyll-*a* values found in the undrained ponds in the second and third years of the study indicate phosphorus enrichment, as phosphorus is generally recognized to be the key nutrient in fish pond fertility (Boyd 1982). This phosphorus enrichment apparently resulted from sediment uptake and re-release of phosphorus. Sediments adsorb phosphorus, and phosphorus regeneration rates are proportional to phosphorus contents of sediments (Chiou and Boyd 1974). This contention is also supported by the observation that phosphorus concentrations were equally low in waters of both treatments at the beginning of each growing season (Figures 2, 3). Hence, phosphorus did not accumulate in waters of undrained ponds.

In summary, the findings suggest that phos-

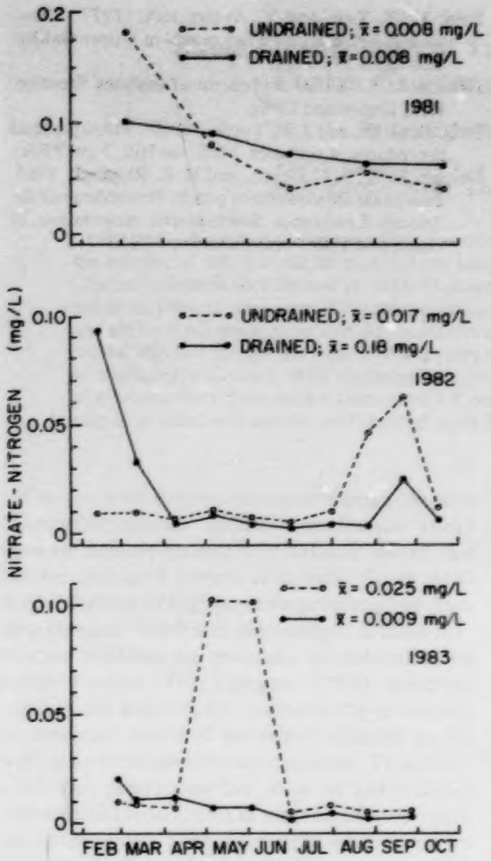

FIGURE 5.—Nitrate-nitrogen concentrations in fertilized sunfish ponds, 1981–1983 (x̄ = mean).

TABLE 1.—Concentrations of acid-extractable phosphorus and organic nitrogen in sediments from four undrained, fertilized ponds and from four fertilized ponds that were drained annually. Means for each year were statistically analyzed by *t*-tests (horizontal comparisons only); asterisk denotes *$P < 0.05$.

		Treatment		
Year	Season	Drained	Un-drained	*t*
		Acid-extractable phosphorus (‰)		
1981	Spring	14.5	17.2	0.50
	Fall	28.9	41.8	2.40
1982	Spring	30.6	40.6	1.65
	Fall	25.1	36.8	1.88
1983	Spring	36.5	39.4	0.39
	Fall	29.0	50.2	3.31*
	After draining	27.5	54.9	4.16*
		Organic nitrogen (%)		
1981	Spring	0.06	0.08	0.94
	Fall	0.16	0.20	0.88
1982	Spring	0.20	0.17	1.08
	Fall	0.21	0.19	0.39
1983	Spring	0.21	0.27	1.79
	Fall	0.26	0.35	1.72
	After draining	0.31	0.39	1.43

phorus availability in undrained, fertilized ponds increases over time. When fertilized at the same phosphorus rate, undrained ponds with a history of phosphorus fertilization will have greater phytoplankton production—and potential for fish production—than fertilized ponds that are drained annually. Pond fertilization rates are usually established from research conducted in ponds which are drained annually. Hence, information reported above suggests that phosphorus fertilization rates can be reduced in undrained ponds with a history of fertilization. Additional research to develop quantitative relationships among phosphorus fertilization history, phosphorus availability from sediments, and fish production would require much time and effort. Meanwhile, pond managers can resort to trial and error methods to ascertain if fertilization may be reduced in particular ponds. For example, phosphorus fertilization rates could be reduced by 10 to 25% and the influence on phytoplankton abundance ascertained. Decreases in phytoplankton abundance will normally reduce turbidity and increase Secchi disk visibility (Almazan and Boyd 1978). If lowering the phosphorus rate does not alter turbidity and Secchi disk visibility, fish production should not be affected.

Acknowledgment

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Rapid Identification of Triploid Grass Carp with a Coulter Counter and Channelyzer[1]

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Abstract. — A method of electronically estimating ploidy in fishes has been developed to maximize the number of fish that can be analyzed per hour while minimizing costs. This technique uses a Coulter Counter Model ZM and a C-1000 Channelyzer to analyze distribution of erythrocyte nuclear volumes. Diploid grass carp (*Ctenopharyngodon idella*) possess mean nuclear volumes of 10.06 μm^3 (SD = 0.60) while mean triploid nuclear volumes were 14.82 μm^3 (SD = 0.96). The technique proved effective for fish that were 27 d to 3 years old, 19–905 mm total length, and rapidly growing or nutritionally stressed. With the modifications and techniques suggested, 1,600–2,400 fish can be evaluated by a three-person team in an 8-h period. Use of this technique allowed triploid grass carp to be efficiently selected and certified prior to stocking for weed control purposes.

Concern over deleterious impacts that grass carp (*Ctenopharyngodon idella*) reproduction could have on aquatic ecosystems curtailed use of this fish for biological control of aquatic plants. Artificial induction of high percentages of triploid grass carp (Malone 1984) renewed interest in these fish, because triploids are generally considered to be sterile (Purdom 1983; Thorgaard 1983). However, some fertile diploids are inadvertently produced; to eliminate potential spawns of diploids in the wild, only triploids should be stocked. To accomplish this, production fish must be individually screened and federal and/or state certification processes instigated. Such a program will require an accurate, quick, and cost effective method of determining ploidy. Production of sterile fish by means of steroids or radiation will not resolve the problem unless an efficient method of certifying sterile fish can be developed.

Although karyotyping remains the only definitive method of determining precise chromosome numbers and configurations (Beck et al. 1980), it is a laborious process which requires considerable skill. Several simpler and quicker methods of accurately estimating ploidy also exist. These include measurement of deoxyribonucleic acid (DNA) content (e.g., DNA fluorography, flow cytometry), measurement of cell or nuclear dimensions (performed microscopically with an ocular micrometer), or phenotypic examinations especially of hybrids where genetic dosage differences from maternal and paternal parents can be evaluated (e.g., electrophoresis [Magee and Philipp 1982], isoelectric focusing, meristics, morphometrics).

Quantification of DNA per cell is effective because triploids contain 1.5 times the quantity of DNA per cell that diploids possess (Rasch et al. 1970; Allen 1983). However, the necessary equipment typically costs over $35,000 and a four-person team generally could not run more than 800 samples in an 8-h period (S. Allen, personal communication).

Similarly, nuclear-size analyses are based on the fact that triploid nuclei, with the extra set of chromosomes, are approximately 50% larger than nuclei of similar diploid cells (Fankhauser 1945; Swarup 1959). Erythrocytes or red blood cells (RBCs) are easily sampled in fish and are relatively consistent in size; their use for this type analysis is well established (Swarup 1959; Cherfas 1966; Cimino 1973; Valenti 1975; Sezaki et al. 1977; Allen and Stanley 1979; Lemoine and Smith 1980; Wolters et al. 1982; Beck and Biggers 1983). Moreover, ploidy is better differentiated by RBC nuclei than by the entire cell, at least in grass carp × bighead carp (*Hypophthalmichthys nobilis*) hybrids (Beck and Biggers 1983). Although microscopic measurement of stained RBC nuclei was the method of choice for simple and relatively inexpensive analyses of hybrid grass carp ploidy, shortcomings of that technique included the time delay associated with fixing, staining, and drying the slides; inconsistent blood smear quality; time involved in measuring an adequate number of nuclei; impracticality of randomly selecting cells for

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[1] Contribution 1 of the Fish Culture Research and Development Program, Post Office Box 1903, Eustis, Florida 32727.

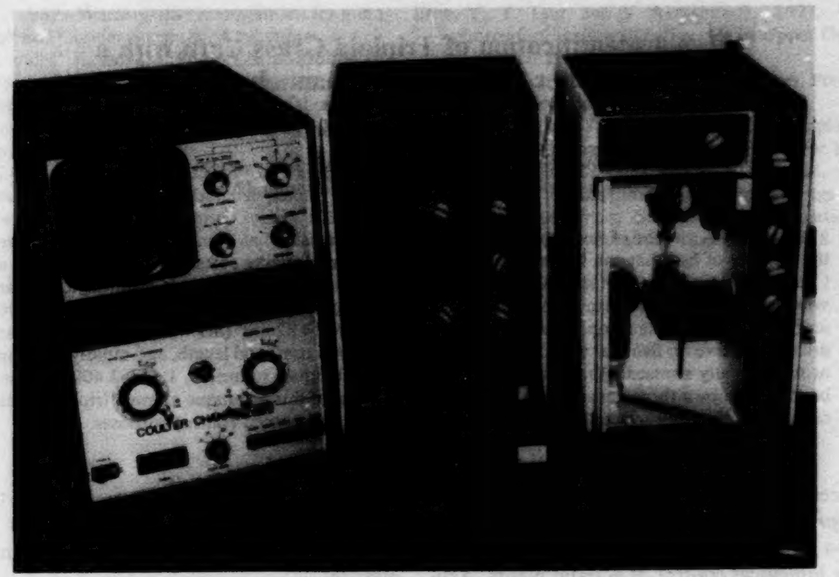

FIGURE 1.—The unit on the left is a Coulter C-1000 Channelyzer—note the overlay in place on the oscilloscope. The other two units combined are the Coulter Counter Model ZM; the central unit is the electronics box and the unit on the right is the sample stand.

measurement; and possibility of technician error in measuring blood cells other than erythrocytes. Therefore, it was considered inadequate as a basis for selecting and certifying triploid grass carp.

The possibility of automating the process by electronically analyzing erythrocyte nuclear volumes was investigated beginning early in 1982. A preliminary comparison of 28 fish (including diploid grass carp, and diploid and triploid hybrid grass carp) resulted in complete agreement between ploidy analyses obtained by measuring RBC nuclei manually with an ocular micrometer and electronically with a Coulter Counter Model ZM and C-1000 Channelyzer. Subsequent trials compared analyses from a Coulter counter, RBC measurements, electrophoresis, flow cytometry, and in vivo karyotypes and determined that the Coulter counter with channelyzer was as accurate as any other method and was simpler and quicker (R. Wattendorf, N. Files, X. Hartmann, and D. Philipp, unpublished data).

Effective use of the Coulter counter and channelyzer for screening individual grass carp for ploidy and for certifying shipments of grass carp as triploid with 100% certainty required that cost be minimized and speed maximized. This paper reports the methodology now being used by the Florida Game and Fresh Water Fish Commission to evaluate production of triploid grass carp, to select triploid individuals from their production fish for stocking, and to certify shipments of grass carp from other sources as triploids with 100% certainty.

Methods

Coulter counter theory.—A Coulter counter (Figure 1) operates by passing particles, which are less conductive than an electrolyte solution in which they are suspended, through an appropriately sized orifice in an aperture tube. The orifice provides the only route via which electrical current can flow between an electrode grounded inside the aperture tube and one which is live on the outside of the tube. These electrodes provide a constant current flow; when a particle is pulled through the orifice by a vacuum, the change in resistance is amplified and accumulated by the Coulter counter. This change in resistance is proportional to the volume of electrolyte displaced and hence to particle size. Each particle can be recorded as an observation to one of 1,000 channels by adding a channelyzer to the system; each channel represents a discrete size range. The channelyzer has an oscilloscope which produces a volume-frequency

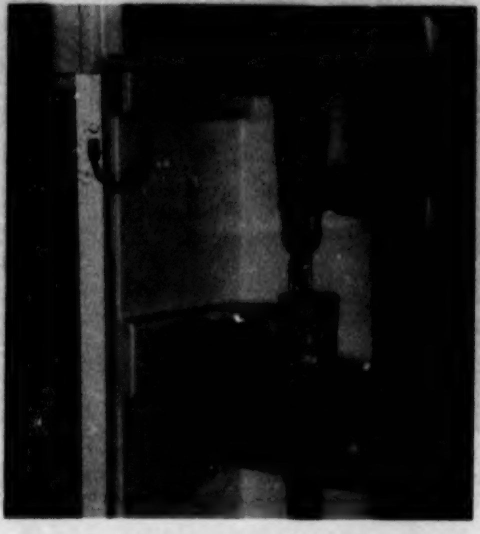

FIGURE 2.—Close-up view of the aperture tube with the orifice immersed in a sample. Note the electrode wrapped around the outside of the tube and the guide.

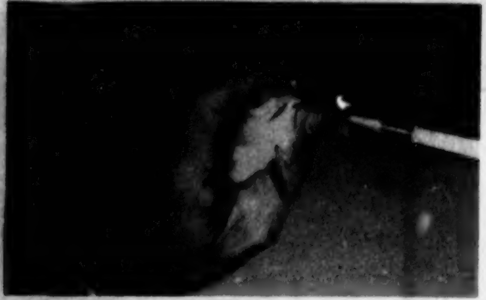

FIGURE 3.—Drawing a 1-μL sample of blood from the branchial artery of a grass carp. Prior to the taking of this picture the artery was pierced with a lancet.

curve for the particles being analyzed. The size range for each channel can be calculated by careful calibration with standard particles.

Modifications.—Several minor modifications have been made to the system to protect the fragile aperture tube from damage and to accelerate the process of analyzing ploidy. A plexiglass guide was constructed and placed on the sample stage to allow the sample vial to be accurately and rapidly positioned. A piece of 1.3-mm-thick styrene was affixed to the base of the guide so the vial would slide smoothly into place (Figure 2). A piece of 30-mm-long silicon tubing was slit lengthwise and placed on the sample-stand riser guide to act as a stop; once in position the stand could be raised rapidly without concern over breaking the aperture tube. A 2-L Erlenmeyer flask with a rubber stopper was placed in series between the sample stand and waste reservoir, this prevented the waste reservoir from filling up too rapidly and increased the vacuum reservoir, which allowed more rapid sampling. A circle of 1.3-mm-thick styrene with a 130-mm diameter was affixed over the oscilloscope, and the graph's axes were traced. Once the ranges of diploid and triploid modal nuclear volumes were known, they were depicted on the overlay (i.e., red for diploid, green for triploid) so that results could be interpreted instantly. A horizontal line corresponding to the smallest triploid nuclear volume was drawn on the ZM monitor to aid in rapid identification of triploids. A magnet was used to override the shut off switch in the doorjamb, which prevented having to open and shut the door between samples. An extra vacuum pump can be added into the vacuum line for maximum speed (J. Lopez-Bernal, personal communication).

Sample preparation.—Each Accuvette sample vial was filled with 10 mL of Isoton II electrolyte and one drop of Zapoglobin lysing agent. (When 1,000 or more samples were to be analyzed within a month, 30 mL of lysing agent were added to each 10 L of electrolyte prior to dispensing.) An automatic system to dispense 10-mL aliquots was used to save time.

About 1 μL of blood was then added to each vial. Samples kept at room temperature can be tested immediately but should be tested within 3 h for best results. Blood dispensed and lysed in the electrolyte can be stored in a refrigerator (5°C) up to 36 h, if warmed to room temperature prior to analysis. Blood was stored for longer periods by drawing it into an EDTA-coated vacutainer, adding an equal volume of EDTA (0.01 M, standard solution), and storing it at 5°C. This blood was diluted with electrolyte and lysed immediately prior to analysis and provided reproducible results for several weeks.

Blood sampling.—Grass carp longer than 70 mm total length (TL) were consistently kept alive after bleeding. Fish were anesthetized with 10–20 mg/L quinaldine; they were bled either by hyperextending the head (tilting it back) and inserting a blood lancet into the tuberosity that rose over the juncture of the gill arches at the isthmus (Figure 3) or by inserting the lancet under a lateral line scale at the caudal peduncle. About 1 μL of blood from

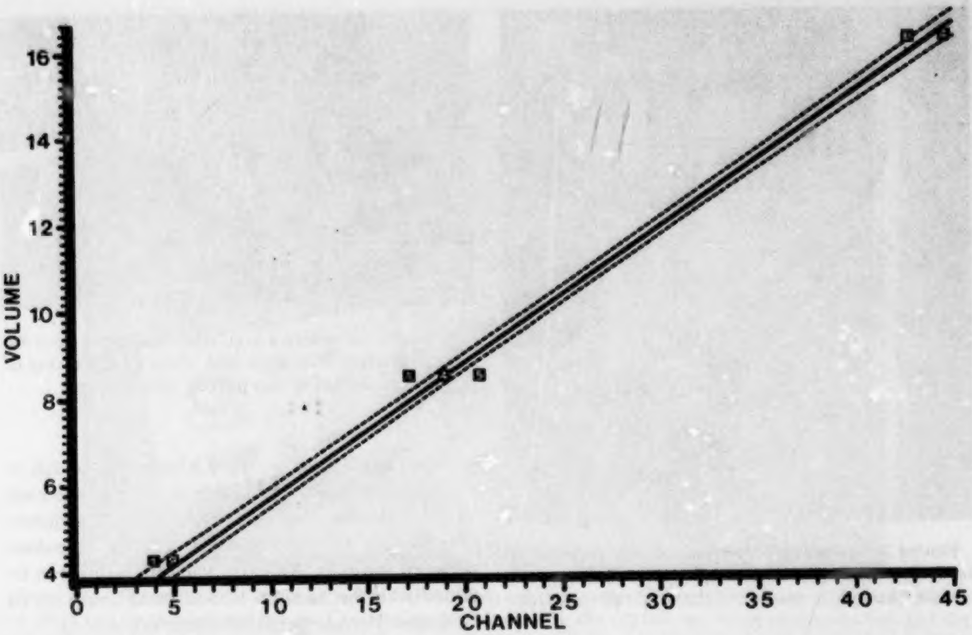

FIGURE 4.—Regression line (solid line) showing the values attained for latex standards along with the 95% confidence intervals (broken line). Numbers indicate the frequency with which a standard appeared in a particular channel based on 10 trials with 100,000 particles counted in the modal channel each time.

the pierced branchial or caudal artery was then sampled with a micropipettor. A positive displacement pipettor could be used and thoroughly rinsed with EDTA between samples. However, we used a quicker, though more costly, method—a micropipettor with disposable, nonwetting polypropylene tips. In either case, blood was dispersed into the electrolyte–lysing agent immediately.

Fish individually screened to produce a 100% triploid population were either kept separate and numbered until the sample was analyzed or were tested immediately. Generally, one person operated the Coulter counter and pipettor; the other two people picked up fish, pierced them, and held them until the analyses were completed. Triploid fish were then placed in an aerated tank containing 100 mg/L Furacin; diploids were rejected. A fourth person was occasionally used to move fish and keep supplies available.

It was possible to obtain an adequate blood sample from the caudal vein of a fish as small as 18 mm TL after caudal amputation. In Florida, grass carp attained this size in about 14 d; however, at that age the blood apparently contained a high percentage of hemoblasts and immature erythrocytes that had nuclei larger than mature erythrocyte nuclei. Therefore, in Florida it is currently necessary to wait an additional week or two, until blood cells mature, before sampling. It may be possible to correlate blood cell nuclear volume to ploidy in very young fish because sizes seem to be consistently larger for both diploids and triploids, but this has not yet been confirmed by karyotyping.

Calibration.—The Coulter Counter Model ZM was set up with a 70-μm-orifice aperture tube. The unit was then precalibrated, with latex microspheres, by the half-count method described in the owner's manual. Once precalibration was completed, the optimum settings for the counter and channelyzer were determined. Although these will vary slightly among machines, the following settings were used for the counter: current, 150; full scale, 10 mA; polarity, negative; lower threshold, 7.0; upper threshold, 99.9 (out); attenuation, 1; and preset gain, 1. The Coulter Channelyzer C-1000 was used with the following settings: base channel threshold, 5.0; and window width, 50.0.

Final calibration was then attained by determining the peak channel in which 4.32-, 8.48- and 16.21-μm^3 spheres occurred on 10 runs each. The modal channel for each run was then regressed

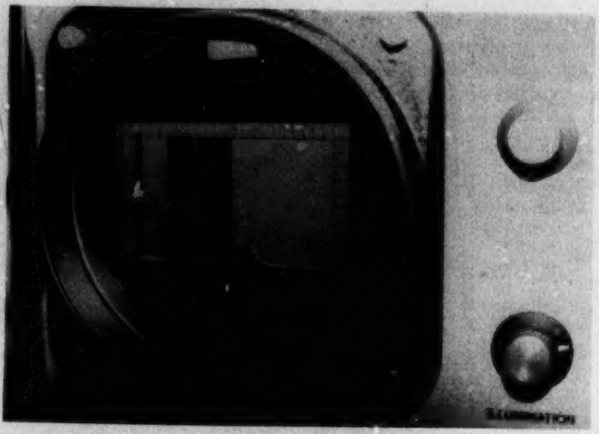

FIGURE 5.—Close up of oscilloscope monitor on the C-1000 Channelyzer, which shows the colored overlay in place (left area is red and right green), with a typical distribution of triploid erythrocyte nuclear volumes displayed. The vertical axis is a frequency count and the horizontal axis is volume.

(Figure 4) on the standard size and the resultant equation used to determine the particle size recorded in each channel was:

$$\text{Volume} = 2.6809 + (0.3061 \times \text{channel});$$

$r = 0.997$.

These settings were designed to position the peak channel of the triploid erythrocyte nuclear volume-frequency curve near the center of the oscilloscope, while keeping diploid peaks fully displayed on the monitor. Enough channels were also displayed to show any haploid or tetraploid nuclei that might have occurred. A mixture of 4.32-, 8.48- and 16.21-μm^3 latex spheres was used as a standard before and after samples were analyzed to assure the unit remained in calibration and was functioning properly. The 16.21-μm^3 particles appeared as large triploid particles and the 8.48-μm^3 spheres simulated small diploid nuclei on the oscilloscope. The standards consistently showed up in the same channel (±2) each time they were examined. In addition, before samples were analyzed a background count was determined on the electrolyte and lysing agent solution; with the manometer select switch at 100 μL, a count of less than 240 particles was considered acceptable.

Analyses.—The vial containing the lysed blood cells was placed on the sample stand and raised into place so that the aperture tube was immersed in the solution. The orifice stopcock was then opened (when samples were analyzed continuously, this stopcock was left open); when particles began passing through the aperture tube, as indicated by vertical pulses on the ZM monitor, the orifice monitor was checked to assure no blockage had occurred. When the monitor was clear and the nuclear volumes appeared homogeneous, the ZM monitor was used to determine ploidy; if there was any doubt, the reset and start buttons on the channelyzer were pressed, allowing the volume-frequency curve to accumulate. A colored overlay could be used to distinguish ploidy based on the presence of the modal peak in the red (diploid) or green (triploid) area (Figure 5); if no overlay was used, the peak channel was converted to volume and the ploidy decision was made on this basis. One hundred particles in the peak channel were adequate for most samples; however, if the distribution was not sufficiently smooth or appeared borderline, the count generally was increased to 1,000 nuclei in the peak channel. Counting more nuclei produced cleaner curves and more reproducible results but also took substantially more time. The curves were plotted on the X–Y recorder when a hard copy was desired (Figure 6).

It was not necessary to clean the aperture tube between samples; most blockages were cleared by immersing the aperture tube in Isoterge detergent and lightly brushing the orifice with a camel hair brush. The aperture tube was soaked in 50% bleach (2.7% sodium hypochlorite) after about every 10,000 samples to reduce the accumulation of organic material around the orifice. Major obstructions were removed by soaking the aperture tube in aqua regia or with an ultrasonic bath.

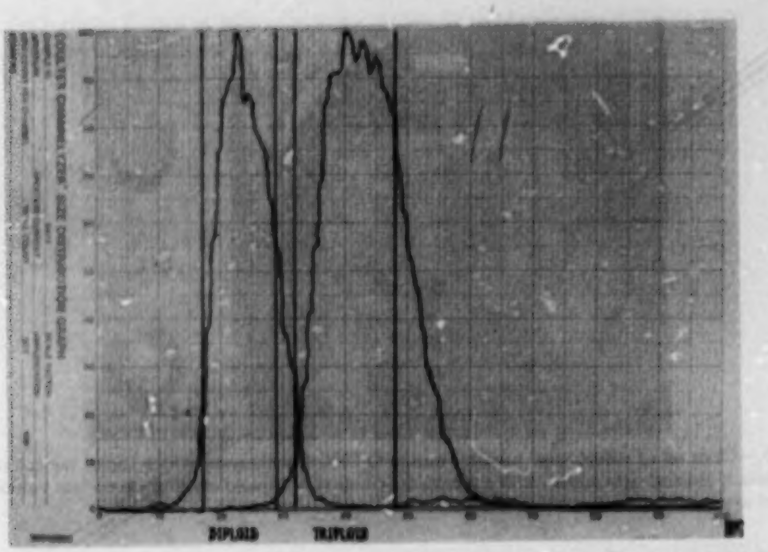

FIGURE 6.—Plot made by an X–Y recorder showing typical nuclear size distributions of diploid and triploid erythrocytes. Vertical lines correspond to the colored overlay on the oscilloscope. The diploid nuclei have a modal size of 9.72 μm^3 shown in channel 23, while the triploid mode occurred in channel 40 (14.92 μm^3).

Results

Range Determination

Analysis of 1,000 grass carp (500 diploid and 500 triploid) demonstrated a clear dichotomy between diploids and triploids (Figure 7). The fish ranged from 27 d to about 3 years old and from 19 to 905 mm TL. All diploids had modal peaks in channels 19–30, representing volumes of 8.5–11.9 μm^3 (mean ± SD, 10.06 ± 0.60 μm^3). The triploid group had modal peaks in channels 32–52, representing volumes of 12.5–18.6 μm^3 (mean, 14.82 ± 0.96 μm^3). Dipioid nuclei were more consistently sized and provided a more spike-like peak than triploid nuclei, which averaged 47% larger.

Assuming random sampling and a normal distribution, 99.5% of the population will occur within 2.81 standard deviations of the mean. Thus 99.5% of all diploids should have nuclear volumes with a mode between 8.32 and 11.75 μm^3 and a similar proportion of the triploids should have a modal nuclear volume between 12.12 and 17.52 μm^3 (Snedecor and Cochran 1976). It was therefore decided that, for certification purposes, fish whose erythrocyte nuclei peaked in channels 19–29 (8.5–11.6 μm^3) were diploids and those with the mode in channels 32–52 (12.5–18.6 μm^3) were triploids. Channels 30 and 31 (11.86–12.17 μm^3) were considered questionable and would not be acceptable, however, in some 75,000 samples, no fish had erythrocyte nuclei whose modal volume fell consistently in channel 31. The few fish whose nuclei showed peaks in channel 30 when 100 nuclei were counted in the peak channel had distributions skewed to the right. By cleaning the orifice and increasing the sample size to 1,000 nuclei in the peak channel these samples shifted into the normal diploid range.

Consistency

There were also concerns about the effects that repeated analyses of blood from an individual fish or that prolonged stress would have on erythrocyte nuclear volumes. To test these concerns, five diploid and five triploid fish, which had been growing rapidly, were given identifying fin clips and were maintained in a raceway for 12 weeks without food. It was hoped that this would induce an anemic condition. During this period each fish was sampled six times (Table 1), and in every case ploidy was correctly evaluated.

Cost Analyses

The initial cost of a Coulter Counter Model ZM and C-1000 Channelyzer with accessories was about $18,000 in 1984. A new model channelyzer, C-256, will soon be available and should provide similar results, possibly at a slightly reduced price.

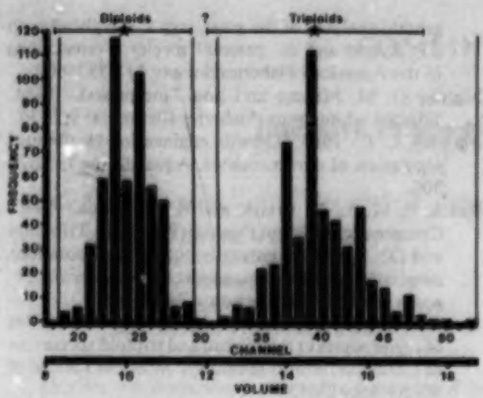

FIGURE 7.—Histogram showing channel modes and corresponding nuclear volume distributions (μm^3) for 500 diploid and 500 triploid erythrocytes. Vertical dashed lines delineate the channels considered as diploid and triploid. Stars over the distributions represent the means of 10.06 for diploids and 14.82 for triploids. Each horizontal line represents the mean ± 2.81 × SD, which is expected to include 99.5% of the diploid or triploid population.

Because large numbers of fish were analyzed, the system was considered cost efficient. A three- or four-person team could examine at least 1,600 fish in an 8-h period, thus labor costs were low compared to other techniques; the cost of supplies per sample was also low. Based on list prices (Curtin Matheson Scientific, 1982), Isoton II and Zapoglobin together cost about 1.5¢ per sample. Accuvettes, which were made to be disposable, cost 7¢ apiece, but in our procedure, they were cleaned, rinsed with distilled water, and reused. The plastic pipette tips cost 3.6¢ apiece; however, positive displacement type pipettors could have been used and rinsed between samples to eliminate this cost. Thus typical costs, including labor and expendable supplies, were 8 to 20¢ per fish, and these costs could be lowered by purchasing supplies in bulk and by using homemade or generic reagents and cheaper labor.

Discussion

The Coulter Counter Model ZM with C-1000 Channelyzer provides a rapid, efficient method of estimating ploidy in grass carp that is independent of fish age (27 d to 3 years), size (19–905 mm TL), and nutritional status. Production spawns can be evaluated for percent triploidy when juveniles are more than 3 weeks old which, in turn, allows researchers and hatchery managers to make appropriate use of grow-out ponds. The rapidity with which fish can be analyzed makes it feasible to analyze every fish before stocking to assure that only triploid fish are used. Moreover, it provides the basis for a sound certification program to assure that producers are supplying only triploid fish.

TABLE 1.—Results from six samplings each of five diploid and five triploid grass carp over a 12-week period during which they were not fed. Values are based on modal volume of erythrocyte nuclei in μm^3.

Ploidy	Mean ± SD	Range
Diploid	9.67±0.30	9.41–10.33
Diploid	9.82±0.32	9.41–10.33
Diploid	9.87±0.26	9.72–10.03
Diploid	10.08±0.23	9.72–10.33
Diploid	10.13±0.32	9.72–10.64
Triploid	14.62±0.27	14.31–14.92
Triploid	14.72±0.37	14.31–14.92
Triploid	14.82±0.57	14.01–15.54
Triploid	15.39±0.53	14.62–15.84
Triploid	16.00±1.00	14.01–16.76

The applicability of the Coulter counter does not stop with grass carp; it can be used with any species when questions of ploidy arise (Benfey and Sutterlin 1984; Benfey et al. 1984). For instance, the recent discovery of naturally occurring triploids in several fish populations has stimulated interest in the frequency and evolutionary role of polyploid fish in nature. Management applications for triploid fish, which revolve around being able to evaluate their ploidy effectively, include: preventing excessive spawning and subsequent stunting in hatchery ponds (Valenti 1975); improving growth of mature hatchery fish by reducing gonadogenesis (Wolters et al. 1982); and delaying mortality in one-time spawners (Allen and Stanley 1979).

Acknowledgments

Steve Ayers and Jorge Lopez-Bernal of Coulter Electronics were instrumental in teaching me the fundamentals of Coulter particle size analyses. Rue Hestand, Boyd Thompson, Jeff Underwood, and the Malones helped prove that the idea could be used on a management scale; Scott Hardin helped with the statistics. To these and all others who helped with developing the technique and critically reviewing this paper I offer my sincere appreciation.

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COMMUNICATIONS

Intensive Production of Guadalupe Bass

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Abstract.—A study was conducted to determine if the threatened Guadalupe bass (*Micropterus treculi*) could adapt to fish hatchery production. Five hormone-injected fish (one female and four males) stocked in a 0.04-hectare spawning pond produced about 1,200 harvestable fingerlings. The fish were size-graded into three classes and stocked into indoor rectangular tanks. Fingerlings, 22–45 mm total length and 0.32–1.02 g, were trained to consume formulated feed in a simulated production environment. They received 1.0–1.5-mm-diameter semi-moist pellets at a rate greater than 48% of the stocked biomass per day throughout the 24-d training period. Training success for the three size classes was 96.4, 97.6, and 100.0%. Growth rates during and following training were similar to those of other *Micropterus* species. These results indicate that the Guadalupe bass will adapt to fish hatchery conditions and can be produced to supplement depleted native populations.

The Guadalupe bass (*Micropterus treculi*) is endemic to the streams draining the northern portion of the Edwards Plateau area of central Texas (Hubbs 1957). In the official checklist of freshwater fishes in Texas, the Guadalupe bass is placed in the depleted category for fishes suffering a large decline in numbers (Hubbs 1976). Guadalupe bass have been classified as "threatened" by the Texas Organization for Endangered Species (TOES 1979) and as "of special concern" by the Endangered Species Committee of the American Fisheries Society (Deacon et al. 1979). Guadalupe bass are rare in various portions of their range due to limited water from isolated source areas, poor water quality, stream impoundments, and, especially, genetic contamination by introduced smallmouth bass (*Micropterus dolomieui*) (Edwards 1978, 1979, 1980).

The present study was conducted during the spring and summer of 1984 in order to determine if Guadalupe bass could adapt to fish hatchery production conditions. Training fish to feed on formulated pelleted feed is important if these fish are to be reared to advanced fingerlings (≥50 mm total length) or to brood fish for future production and stocking.

Methods

Three adult Guadalupe bass were electroshocked from Buchanan Reservoir, Llano County, Texas, on March 27, 1984. All were transported to the San Marcos facility in 3.5 h in a medicated saline hauling solution (Carmichael 1983). They were placed in a covered holding tank overnight before being prophylactically treated for diseases with 5 mg/L $CuSO_4$: 2.5 mg/L citric acid for 1 h. On March 29, they were treated with a 1,000 mg/L salt dip. They were then anesthetized with 200 mg/L tricaine (MS-222) and injected with 4,000 International Units/kg human chorionic gonadotropin. The female—408 mm total length; 900 g—along with two males—231 and 242 mm; 130 and 145 g—was stocked into a 0.04-hectare pond. The pond was mud-lined, had a concrete catch basin and steps, and contained one gravel 0.5 × 0.5-m nest site in 60 cm of water.

Two additional male Guadalupe bass—approximately 230 mm total length; 140 g—were collected from the Llano River, Llano County, Texas, on April 4. They were treated in a manner similar to the other brood fish and were stocked into the pond on April 6.

Lake Buchanan and the Llano River were chosen as the sources for broodfish because no successful introductions of smallmouth bass have occurred in either system (R. J. Edwards and C. Hubbs, personal communication). Largemouth bass (*Micropterus salmoides*), however, were taken at the collection sites.

Fingerlings used in feed-training trials were produced by the traditional open-pond spawning method (Snow 1975). Before pellet training, fingerlings fed on zooplankton and insects in the spawning pond. The pond was treated with 2.0 mg/L Aquazine on May 24 to retard growth of aquatic vegetation (*Chara* sp.). The pond was harvested May 30 and the fingerlings were graded and stocked into three indoor 52-L tanks. Water exchange rate in each tank was 6.5/h. The fingerlings received a static, 1-h, 5-mg/L $CuSO_4$: 2.5-mg/L

TABLE 1.—Stocking data and training results of Guadalupe bass feeding experiments.

Number per tank[a]	Initial weight (g/fish)	Harvest weight (g/fish)	Training success[b] (%)
306	0.32	0.72	96.4
124	0.43	1.28	97.6
65	1.02	2.54	100.0

[a] Number of fish stocked into each tank was initially estimated, but readjusted based on total mortalities removed from the tank plus fingerlings on feed at the end of training.

[b] Number of feeding fish as a percentage of those stocked.

citric acid prophylactic disease treatment on May 31. The fish were maintained in the tanks for 6 d before being regraded and stocked into training tanks; ground fish flesh and Biodiet feed were fed ad libitum during this time.

The character tested was "training success" (Anderson 1974), defined as the number of fingerlings feeding at the end of the training period divided by the number stocked at the beginning of the period. The training diet was Biodiet, a stabilized, semi-moist, formulated diet. Pellets 1.0–1.5 mm in diameter were dispensed from Sweeney automatic feeders. Feed aliquots were dispensed at 5-min intervals between 0800 and 1800 hours daily, 7 d/week. Feeding rates were greater than 48% of the stocked biomass per day. The fingerlings were greatly overfed to provide frequent feeding opportunities and to reduce size variation related to competition. Dead fish were collected and counted daily when tanks were cleaned. The fish were treated on June 8 for 1 h with 5 mg/L $CuSO_4$: 2.5 mg/L citric acid as a disease prophylaxis.

Feeding trials were conducted in a controlled-environment holding house. Culture tanks were rectangular fiberglass tanks, 122 × 30 × 30 cm. The culture volume of each tank was 85 L regulated by a 20-cm standpipe. Fingerlings were crowded by a screen divider into an area 30 × 30 × 20 cm. Water exchange rates were set at 5.0/h. Relatively high exchange rates maintained acceptable water quality and facilitated cleaning. Stocking densities (and feeding rates) were lower than those used during largemouth bass production—2.5–5.0 g/L versus 6.8–7.0 g/L (Williamson 1983). Well water was supplied to each tank individually. Water quality characteristics at inflow were: temperature 23.0–23.5°C; minimum dissolved oxygen 3.5 mg/L (mean 6.5 mg/L); pH 7.0–7.5; free carbon dioxide 20.0 mg/L; bicarbonate alkalinity 320.0 mg/L; and carbonate alkalinity 0.0 mg/L.

The training period was June 7 to June 22. When each test was concluded, feeding fingerlings were counted and the total weight recorded. Nonfeeding fingerlings were counted as mortalities (Williamson 1979). No cannibalism (indicated by battered or regurgitated fingerlings, tails protruding from another's gape, or outsize fingerlings) was observed during the trials. Consequently, the recorded mortalities and feeding fingerlings at the end of training closely approximated the number stocked (>96%). Training success was, therefore, based on the number of feeding fish as a percentage of those stocked (Table 1).

Following training, fish were graded by size into three groups and placed in culture tanks. All fish were fed 1.5–2.5-mm-diameter Biodiet pellets ad libitum until August 13. The groups were again graded by size and one group (318 fish; mean = 6.59 g/fish) was placed into a 0.04-hectare culture pond. A 2-m^2 floating plastic sunscreen was placed in the middle of the pond in an attempt to provide common cover so that the fish would aggregate. The fish were fed 3–4-mm-diameter Biodiet until September 7 and then 5-mm pellets until October 9.

Results and Discussion

Limited observations of the adult fish in the spawning pond indicate that these fish have a more secretive and cryptic nature than largemouth bass. Fish were never seen together and when they moved they darted quickly. Edwards (1980) concluded that larger individuals of the *Micropterus* genus establish and defend feeding territories in the wild. Under hatchery conditions, largemouth bass aggregate and separate only during breeding season. In this aspect, the Guadalupe bass differ from largemouth bass, remaining stationary near the pond bottom close to any available cover such as the catch basin, vegetation, and depressions in the pond bottom. Guadalupe bass spawn near slowly to moderately flowing water in the wild—backwater with water inflow (Edwards 1980). No spawning or courtship behavior was observed and no males were seen clearing or tending the nest site before fry were observed. Edwards (1980) reported parental care of the nest by wild male Guadalupe bass when pond temperatures ranged from 20 to 24°C.

A small school of fry, estimated at 200 individuals, was observed near the concrete steps in the pond 30 d after adult fish were stocked. The fry appeared to be 10–15 d old based on a size comparison with largemouth bass fry. A second school, estimated at 1,000 individuals, was observed near the gravel nest site 35 d after stocking. Those fry appeared to be 4–9 d old, again compared to largemouth bass. The aggregations of fry dispersed 4–10 d after they were sighted, rather more quickly than largemouth bass fry, which may stay aggregated for well over 30 d. Approximately one-third of the fingerlings died during harvest because the cryptic fish stayed in the vegetation and would not follow the receding water as the pond was drained, even though workers tried to chase them into the catch basin. About 900 fingerlings were transported to the holding house and about 500 of these were used for training experiments. The number of fingerlings accounted for, about 1,300–1,400 from at least two spawns, indicated that a female will spawn more than once during a breeding season. Edwards (1980) reported that individual female Guadalupe bass had 399–9,286 ova in their ovaries. The number of fingerlings produced in our study falls within that range.

Training success was high for the sizes of fingerling Guadalupe bass that we tested (Table 1). Past studies with species of *Micropterus* have shown training success to be highly variable. Williamson (1983) reported training successes of 23.5 to 43.1% for two strains of largemouth bass that were close to the size of our Guadalupe bass fingerlings. Flickinger and Smeltzer (1983) reported 88.5 to 89.2% training success for spotted bass (*Micropterus punctulatus*) that were slightly larger (1.04 g). Anderson (1974) reported 14.0 to 80.2% training success for smallmouth bass of a similar size. Williamson (unpublished data) recently obtained training success at 80 to 98% for four strains of largemouth bass. The Guadalupe bass appeared to train as well as, or better than, largemouth bass.

Size of fingerlings at training is a primary factor contributing to successful training (Anderson 1974; Snow 1975; Williamson, unpublished data). The size of stocked Guadalupe bass did affect training success—within the sizes that we tested (Table 1). Largemouth bass that are 30 mm long, and 0.40–0.50 g are considered large enough for training (Williamson 1983). Smaller largemouth bass fingerlings are more difficult to handle and train. Guadalupe bass apparently do not have the extreme cannibalistic behavior that largemouth bass possess.

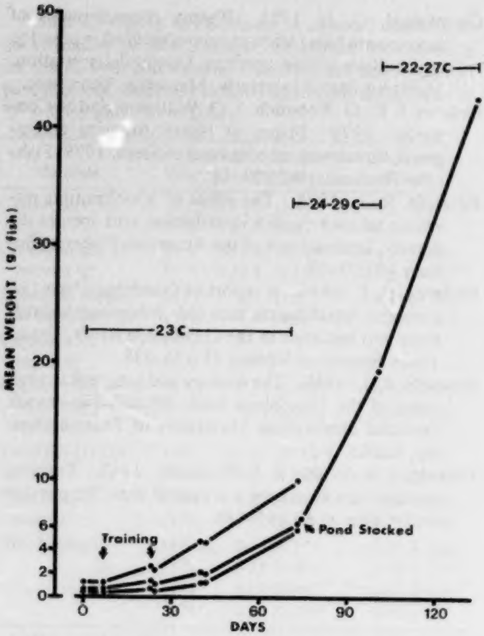

FIGURE 1.—Mean weights of three size-graded groups of Guadalupe bass fed pelleted feed from May 30 to October 9, 1984.

Increases in mean weight of the Guadalupe bass fingerlings are presented in Figure 1. The growth rates of Guadalupe bass are similar to those of largemouth bass (Williamson 1983; Williamson, unpublished data). Edwards (1980) reported growth rates of a wide range of sizes of Guadalupe bass to be 0.43–0.91 mm/d. The fish in this study grew 0.36–0.54 mm/d during the training period and at that rate or higher during the pond growout period. Guadalupe bass fingerlings fed aggressively like largemouth bass fingerlings.

The results of this study show that Guadalupe bass can be raised at fish hatcheries. Behavioral characteristics make them more difficult to harvest from ponds than largemouth bass. Fingerling Guadalupe bass can be successfully trained to consume formulated diets.

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Corbicula as a Biological Filter and Polyculture Organism in Catfish Rearing Ponds

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Abstract.—*Corbicula fluminea*, an introduced Asian clam, was stocked with channel catfish (*Ictalurus punctatus*) in Illinois culture ponds. Survival of the stocked clams was 36–79% over summer, but reproduction was poor and the populations declined. Nevertheless, ponds with *Corbicula* had less dissolved oxygen depletion, lower turbidity, and greater primary production than ponds without the clam. Growth of channel catfish was unaffected by the presence or absence of clams. *Corbicula fluminea* has potential value as a polyculture organism and as a biological filter where water temperatures do not exceed 30°C.

Corbicula fluminea (a clam introduced from Asia) has been viewed commonly as a liability in the United States (Sinclair and Isom 1963; Eng 1979). However, many of its features indicate potential as a biological filter and polyculture organism. *Corbicula* can survive, grow, and reproduce in fish ponds (Buttner and Heidinger 1980; Buttner 1981; Buttner, in press). It is more tolerant of commonly used aquaculture chemicals than most fishes (Chandler and Marking 1979). *Corbicula* exhibits high fecundity, has a prolonged period of reproduction, and lacks the parasitic glochidia stage characteristic of unionids (Sinclair and Isom 1963; Britton et al. 1979). *Corbicula* has a high filtration rate (Buttner and Heidinger 1981), can effectively harvest detritus, bacteria, and phytoplankton (Sinclair and Isom 1963), and exhibits rapid growth (Britton et al. 1979; Buttner and Heidinger 1980). The soft tissue of *Corbicula* is approximately 45% protein by dry weight (Haines 1979), and commercial markets exist for the clam as fish bait, for use in the aquarium trade, as food for domesticated animals, and for human consumption (Chen 1976; Sickel et al. 1981; Britton and Sickel 1982).

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The potential of *Corbicula* as a biological filter and polyculture organism was evaluated, and the results are presented in this paper.

Methods

Combinations of clams and channel catfish (*Ictalurus punctatus*) were evaluated in four 0.06-hectare earthen ponds located in Jackson County, Illinois, during 1977 and 1979. During both years two test ponds received *Corbicula* and two control ponds received no *Corbicula*; fingerling channel catfish were stocked in all four ponds. In 1977, clams of 10 to 42 mm shell length were stocked in two ponds at 828 and 1,010 kg/hectare; in 1979, clams were again stocked in the ponds at 1,222 and 1,717 kg/hectare. In April 1977, each of the four ponds was stocked with 300 channel catfish fingerlings averaging 49 g; in May 1979, each pond again received 302 fingerlings averaging 60 g. In both years, catfish were fed number 6 Purina Trout Chow at 2% body weight daily, 6 d/week. Feeding rates were adjusted weekly and all ponds received equivalent quantities of feed. Dissolved oxygen, net diurnal production, water temperature, pH, turbidity, alkalinity, and nitrogenous wastes were monitored; survival and reproductive success of *Corbicula* were determined; and effect of *Corbicula* on catfish survival, growth, and feed conversion was examined (Buttner 1981; Buttner, in press). Alkalinity averaged 84 mg/L and pH averaged 7.4. Water temperature at dawn averaged 25°C (range 15–32°C) and at dusk averaged 28°C (range 18–34°C); dissolved oxygen at dawn approximated 50% saturation and by late afternoon approached or exceeded 100% saturation. Ponds were harvested and drained in October 1977 and 1979. Unless stated otherwise, all analyses were conducted using the GLM package of the Statistical Analysis System (Helwig and Council 1979).

Results and Discussion

Corbicula survived and reproduced in catfish ponds, but recruitment was not sufficient to maintain stock density. Survival of stocked *Corbicula* averaged 36% in 1977 and 79% in 1979. Reproductive success of *Corbicula* was indicated by the presence of several larval clams less than 0.02 mm in shell length, identified from 10 zooplankton collections taken between August and October 1979. Survival of larval *Corbicula* was poor and very few young-of-the-year clams were collected at harvest. Adult *Corbicula* survived Karmex at 1 mg/L, used to control aquatic vegetation; Batex at 0.25 mg/L, used to eliminate crayfish; and rotenone at 2 mg/L, used to kill contaminant fish.

TABLE 1.—Effect of *Corbicula* on channel catfish and water quality in four 0.06-hectare ponds. Mean values for ponds with and without *Corbicula* are shown (SD in parentheses). Asterisks denote significant differences at $^{*}P < 0.05$ and $^{**}P < 0.01$.

Variable	Year	*Corbicula* present	*Corbicula* absent
Catfish survival (%)	1977	97.3 (1.9)	94.2 (5.2)
	1979	97.4 (0.5)	98.2 (0.0)
Catfish weight at harvest (g)	1977	379.8 (93.8)	394.7 (84.0)
	1979	348.5 (89.5)	367.0 (107.6)
Dissolved oxygen at dawn (mg/L)	1977	5.2 (1.3)*	4.8 (1.3)
	1979	4.3 (1.5)	4.0 (1.5)
Oxygen <3.0 mg/L (d)	1977	7*	13
	1979	16	21
Primary productivity (O_2 mg/L/h)	1977	0.15 (0.10)**	0.11 (0.08)
	1979	0.30 (0.16)	0.29 (0.18)
Turbidity (JTU)[a]	1977	60.4 (27.0)**	79.4 (17.9)
	1979	59.5 (17.0)	66.6 (28.8)
Total NH_3-N (mg/L)	1977	0.23 (0.20)**	0.06 (0.06)
	1979	0.77 (0.50)	1.18 (0.94)
NO_2-N (mg/L)	1977	0.64 (0.54)	0.59 (0.48)
	1979	0.59 (0.40)	0.49 (0.21)
NO_3-N (mg/L)	1977	0.07 (0.06)	0.07 (0.05)
	1979	0.04 (0.03)*	0.03 (0.01)

[a] JTU = Jackson turbidity unit.

No significant differences ($P > 0.05$) in survival or growth of channel catfish were observed between ponds with and without *Corbicula*, but water quality was improved in ponds with *Corbicula* (Table 1). Dissolved oxygen at dawn was greater, incidence of dissolved oxygen below 3 mg/L was lower, rate of primary production was higher, and turbidity was lower in ponds with *Corbicula*. Nitrogenous wastes were sometimes greater in ponds with *Corbicula*. Trends were similar in both 1977 and 1979.

Habel (1970) and Busch (1974) observed that *Corbicula* clams, stocked at 6,860 to 40,860 kg/hectare in 0.0007-hectare pools, were associated with decreased turbidity, increased channel catfish survival, and increased mean weight of catfish. More recently, Haines (1979) observed that turbidity of sewage effluent was lower in the presence of *Corbicula*. However, as in the present study, clam mortality was high and probably was related to an intolerance to elevated temperatures and low oxygen. *Corbicula* uptake of oxygen decreases greatly between 25 and 30°C; at these temperatures the rate of uptake at 70% oxygen saturation is half that in waters at 100% saturation (Mattice and Dye

1979; McMahon 1979). Water temperatures in excess of 33°C produce mortalities (McMahon and Aldridge 1976).

Based on these observations, the greatest potential of *Corbicula* as a biological filter and polyculture organism would be in systems with cooler temperatures and higher dissolved oxygen than commonly found in channel catfish ponds. The clam should be introduced only in waters where temperatures rarely exceed 30°C and dissolved oxygen is greater than 50% saturation. In such a system, *Corbicula* could be stocked on the substratum of earthen ponds or possibly in cages suspended in slowly circulating water. *Corbicula* may be useful in promotion of water quality in discharge canals, after secondary treatment of sewage, or in the effluent of fish raceways. Polyculture with cool water fishes such as striped bass (*Morone saxatilis*), walleye (*Stizostedion vitreum*), sauger (*S. canadense*), and yellow perch (*Perca flavescens*) may be possible because optimum temperatures for these species range from 22°C to 28°C (Hokansen 1977; Coutant and Carroll 1980). These temperatures more closely approximate the optimal temperature for *Corbicula* than does the optimal temperature for channel catfish (30°C: NRC 1977). Prior to extensive use of *Corbicula* in polyculture systems, its effect on nitrogenous wastes should be identified.

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Effect of Reused Water on Brown Trout

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Abstract.—Brown trout fingerlings (*Salmo trutta*) were reared for 141 d in water that passed successively through a series of seven troughs. Fish loading per trough was based on a flow index of 36.4, thus the cumulative index was 254.8 at the seventh water use. Two indicators of water quality were measured after each water use; average dissolved oxygen concentration decreased from 7.6 mg/L after the first water use to 3.6 mg/L after the seventh use, and average total ammonia concentration increased from 0.1 mg/L to about 0.9 mg/L. Growth rate began to decline at the fourth water use, when dissolved oxygen averaged 4.8 mg/L and total ammonia averaged 0.6 mg/L.

The reuse or recycling of water in intensive fish culture is a common practice. Larmoyeux and Piper (1973) studied the effect of reused water on rainbow trout (*Salmo gairdneri*) and demonstrated a decline in fish quality, as evidenced by reduction in growth and by gill damage after fish were held for 230 d in water with less than 5 mg/L dissolved oxygen and more than 0.5 mg/L ammonia. Because salmonids are widely cultured and have high economic importance, the effect of reused water on species other than rainbow trout is of considerable interest to fish culturists.

Ammonia and dissolved oxygen concentrations are of particular concern in hatcheries where water is reused. Burrows (1964) concluded that ammonia was the metabolic product causing gill damage in chinook salmon (*Oncorhynchus tshawytscha*). Severe gill damage and increased incidence of disease in rainbow trout, after long-term exposure to ammonia, have been described by Reichenbach-Klinke (1968), Smith and Piper (1975), and Smart (1976). In the present study, we evaluated the effect of reused water on growth, mortality, food conversion, and cellular changes in fingerling brown trout (*Salmo trutta*).

Methods

Water passed through a duplicate series of seven troughs to simulate a serial water-reuse system (Figure 1). Each trough contained 0.078 m^3 of water. The water supply for the study was a natural ground water spring with pH 7.8 and total hardness 200 mg/L ($CaCO_3$). Water flow through the series of troughs was adjusted to 10 L/min and water temperature was maintained at 10 ± 1°C. Fish loading was based on a metric flow index,

$$F = 0.01 \times \text{mg}/(\text{mm} \times \text{L/min});$$

initial values of fish weight (mg), water inflow (L/min), and fish length (mm) established an F of 36.4 per trough, giving a cumulative flow index of 254.8 at the seventh water use (Piper 1975). Fish were fed a standard commercial trout feed at a rate based on a metric hatchery constant of 27.4 (Buterbaugh and Willoughby 1967).

At 2-week intervals, the fish were weighed to determine growth rate and the number of fish was reduced to maintain a flow index of 36.4 in each trough at the beginning of each 2-week period. From length–weight tables, fish length was cal-

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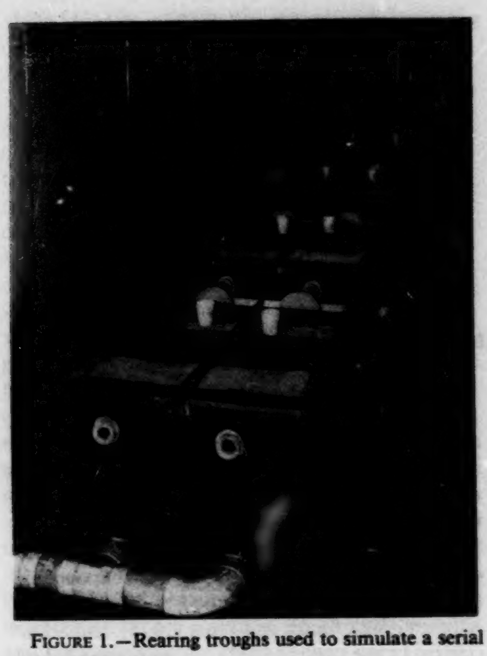

FIGURE 1.—Rearing troughs used to simulate a serial water-reuse system.

culated at the end of each 2-week period based on a condition factor (*K*) of 1.1×10^{-5} (Bowen and Studdard 1970).

Dissolved oxygen and ammonia were measured during the afternoon the day before the fish were weighed. Dissolved oxygen was measured with a YSI (Yellow Springs Instrument, Incorporated) oxygen meter and ammonia was determined by the Nesslerization method (APHA et al. 1971). Samples were taken for histological examination at the end of the study (141 d). Fish were preserved in Bouin's solution; representative tissues (gill, liver, kidney, and intestine) were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

When necessary, fish were treated with 2 mg/L Hyamine 3500 to control bacterial gill disease.

Data were analyzed for significance at the $P <$ 0.05 level by the Student–Newman–Keuls test (Sokal and Rohlf 1969).

Results and Discussion

Growth, mortality, food conversion, and water quality data are shown in Table 1. Average dissolved oxygen concentration decreased from 7.6 mg/L after the first water use to 3.6 mg/L after the seventh use while average ammonia concentration increased from 0.1 mg/L to 0.9 mg/L. As water was reused, the pH decreased from 7.8 to 7.4.

Food conversion increased to more than 2.0 during the fifth water use. Growth rate began to decline during the fourth water use, and was poorest during the last water use. Mortality was highest in the fifth, sixth and seventh water uses. The fish in these troughs were more susceptible to bacterial gill disease and treatment with Hyamine 3500 was necessary after fish had been reared in the reused water for about 8 weeks.

Fish raised in water used six or seven times were thin, their dorsal fins were severely eroded, and their opercula were flared. Gills from fish reared for 141 d in the sixth and seventh water uses showed slight hypertrophy of epithelium and mild fusion of gill lamellae. Other tissues examined (liver, kidney, and intestine) showed neither consistent nor significant cellular changes. These findings are similar to those described for rainbow trout reared

TABLE 1.—Cumulative growth and water quality data for brown trout fingerlings reared in water reused through a series of troughs for 141 d.[a]

Water use	Cumulative flow index $\left(\frac{\text{mg} \times 0.01}{\text{mm} \times \text{L/min}}\right)$	Total length increase (mm)	Total conversion (food fed/ weight gain)	Dissolved oxygen (mg/L)	Total ammonia (mg/L)	Mortality (%)
1	36.4	59 u	1.75 u	7.6 u	0.1 u	0.4 u
2	72.8	59 uv	1.74 u	6.6 v	0.3 v	0.2 u
3	109.2	58 uv	1.79 u	5.7 w	0.4 v	0.2 u
4	145.6	55 vw	1.93 u	4.8 x	0.6 w	0.5 u
5	182.0	51 w	2.32 uv	4.2 y	0.7 x	1.5 v
6	218.4	45 x	2.87 vw	3.8 z	0.8 xy	2.6 w
7	254.8	42 x	3.70 w	3.6 z	0.9 y	3.8 x

[a] Values are means of duplicate troughs. Means within a column not sharing a common letter are significantly different ($P \leq 0.05$).

in reused water for 4 months (Larmoyeux and Piper 1973).

Deterioration of water quality due to serial reuse adversely affected fingerling brown trout. When dissolved oxygen fell below 5.0 mg/L and ammonia increased above 0.5 mg/L, growth rate decreased and food conversion and mortality increased. These values of dissolved oxygen and total ammonia may not cause problems when they occur singly, but may provide a suitable guideline for projecting water requirements.

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TECHNICAL NOTES

Computer Program Calculation of Gas Supersaturation in Water

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Abstract.—A short computer program, written in BASIC for the Apple IIe or IBM PC computer, efficiently performs all the calculations required to determine gas pressure and percent saturation values for water. Input for the program is limited to empirical determinations of barometric pressure, water temperature, differential dissolved gas pressures, dissolved oxygen, and salinity. An optional routine is included for obtaining a printed report of input data and results. The program can be easily modified to run on most other microcomputers that use BASIC programming language.

Nitrogen gas supersaturation is believed to increase the susceptibility of fish to secondary infections and, in severe cases, produces lethal gas bubble disease (Wold 1973). Rucker (1972) and Harvey (1975) provided reviews of gas bubble disease in fish and related its occurrence to physical and chemical laws of solubility. Weitkamp and Katz (1980), who reviewed the literature on gas supersaturation, documented numerous examples of adverse effects of supersaturation on cultured and wild populations of fish.

The preferred method of analysis for relative gas saturation is direct measurement of total gas pressure with instruments described by Fickeisen et al. (1975), D'Aoust et al. (1975), or Bouck (1982), or other similar instruments, all of which are based on the membrane-diffusion method.

Many equations have been developed to calculate the relative pressures and percent saturation of the principal gases that are involved in gas supersaturation (Weiss 1970; Nebeker et al. 1976a, 1976b; Bouck 1982; Colt 1983, 1984). The equations require that empirical determinations be made of barometric pressure, water temperature, differential dissolved gas pressures (saturometer or gasometer reading), dissolved oxygen, and salinity. They also require that values of the water vapor pressure and Bunsen solubility coefficient for oxygen be obtained from tabular values that correspond to the water temperature and salinity. The series of equations is then used to calculate the gas pressure and percent saturation for total dissolved gases, oxygen, and nitrogen, as well as the ratio of percent saturation of oxygen to that of nitrogen. The computer program described here efficiently performs all of the calculations required to determine gas pressure and percent saturation values.

Program Description

Several equations have been proposed that describe the physical and chemical laws of solubility of gases in water. Not all of these equations yield the same values. The differences usually arise from different ways of compensating for water vapor pressure (Colt 1983). The computer program presented here (Appendix A) is based on the equations of Bouck (1982) and Colt (1983) and is written in Applesoft BASIC language for the Apple IIe computer. Appendix B provides a listing of the line changes required to modify the program in Appendix A so that it will run on an IBM PC computer.

The program prompts the user to provide the barometric pressure (mm Hg), water temperature (C), differential dissolved gas pressure (saturometer or gasometer reading, mm Hg), oxygen concentration (mg/L) and salinity (parts per thousand, ppt). In most freshwater situations, a value of zero (0) would be entered for salinity. Values for the Bunsen solubility coefficient and water vapor pressure for the corresponding water temperature and salinity are provided automatically by the computer from equations derived by Weiss (1970) and Weiss and Price (1980), respectively. No correction is made for carbon dioxide because the partial pressure of carbon dioxide is generally so low that it can be ignored (Bouck 1982; Colt 1983).

The results of calculations appear on the screen monitor and are followed by the question "Would you like results printed? (Y/N)." An affirmative reply (Y) instructs the printer to prepare hard copy. An example is shown in Table 1.

The equations of Bouck (1982) and Colt (1983) are in agreement regarding the pressure and percent saturation of total dissolved gases and oxygen. However, Bouck (1982) converts the pressure of

TABLE 1.—Sample output from gas saturation calculation program.

```
**************************************
*     Saturometer Calculations       *
**************************************

SITE= TEST WELL #1
DATE= 10-23-83

INPUT DATA
----------
BAROMETRIC PRESSURE (mm Hg)=..... 762
WATER TEMPERATURE (C.)=.......... 13
SATUROMETER READING (mm Hg)=..... 75
OXYGEN CONCENTRATION (mg/L)=..... 10.2
SALINITY (PPT)=.................. 0

RESULTS
-------
BUNSEN SOLUBILITY COEFFICIENT=... .0357
WATER VAPOR PRESSURE (mm Hg)=.... 11.22

TOTAL DISS. GAS PRESS. (mm Hg)=.. 837
OXYGEN PRESSURE (mm Hg)=......... 151.9
NITROGEN PRESSURE (mm Hg)=....... 665.6
N2 + ARGON PRESS. (mm Hg)=....... 673.9

TOTAL HYPERB. PRESS. (mm Hg)=.... 75
OXYGEN HYPERB. PRESS. (mm Hg)=... -5.3
N2 + Ar HYPERB. PRESS. (mm Hg)=.. 80.4

TOTAL PERCENT SATURATION=........ 109.8
OXYGEN PERCENT SATURATION=....... 96.6
NITROGEN PERCENT SATURATION=..... 113.5

RATIO OF OXYGEN % / NITROGEN %=.. .851
**************************************
```

inert gases to nitrogen gas pressure by multiplying inert gas pressure by 0.9877. Colt (1983) does not make this conversion and refers to this value as nitrogen + argon pressure. Both values are provided by this computer program. Colt (1983) believed that gas pressure should be reported in terms of the difference between the total dissolved gas pressure and the ambient hydrostatic pressure and referred to this difference as the uncompensated pressure or hyperbaric gas pressure. Hyperbaric gas pressure is computed by the program, as well as percent saturation—the unit of measure now being used in water quality standards. Inasmuch as the equation presented by Bouck (1982) for percent saturation of nitrogen is mathematically equivalent to the equation presented by Colt (1983) for percent saturation of nitrogen + argon, only one value for percent saturation is provided in the output from the computer program.

At a given total gas pressure, decreasing the oxygen to nitrogen ratio increases the lethality of the dissolved gases (Rucker 1975; Nebeker et al. 1976a, 1979). The oxygen to nitrogen ratio is included in the printed output as additional information for use in the interpretation of biological effects of dissolved gases.

The program can be easily modified for use on most other microcomputers that use BASIC programming language.

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Appendix A: Program for calculating gas supersaturation (Apple IIe).

```
10 REM ........................SATUROMETER CALCULATIONS.......................
20 REM .....by VERDEL K. DAWSON...................................8-31-83....
30 REM ........NATIONAL FISHERY RESEARCH LABORATORY.........................
40 REM ........LaCROSSE, WISCONSIN..........................................
50 REM .....THIS PROGRAM CALCULATES THE PRESSURE AND PERCENT SATURATION OF
60 REM .....GASES DISSOLVED IN WATER.  THE PROGRAM REQUIRES THE INPUT OF
70 REM .....BAROMETRIC PRESSURE, WATER TEMPERATURE, SATUROMETER READING,
80 REM .....DISSOLVED OXYGEN CONCENTRATION, AND SALINITY. BUNSEN SOLUBILITY
90 REM .....COEFFICIENTS AND WATER VAPOR PRESSURE ARE AUTOMATICALLY CALCULATED
100 REM ....BASED ON WATER TEMPERATURE AND SALINITY.
110 G$ = CHR$ (7)
120 HOME
130 GOSUB 1000
140 REM .....ENTRY OF EMPIRICAL DATA
150 INPUT "ENTER SITE NAME.... ";S$
160 PRINT
170 INPUT "ENTER DATE (ie MM-DD-YY).... ";D$
180 PRINT
190 INPUT "ENTER BAROMETRIC PRESSURE (mm Hg) ";P
200 REM .....CHECK VALIDITY OF ENTRY
210 IF P < 660 OR P > 787 THEN PRINT G$: PRINT "BP LIMITS ARE 660 TO 787": GOTO 190
220 PRINT
230 INPUT "ENTER WATER TEMPERATURE (C.)..... ";T
240 IF T <  - 1 OR T > 40 THEN PRINT G$: PRINT "TEMP. LIMITS ARE -1 TO 40": GOTO 230
250 PRINT
260 INPUT "ENTER SATUROMETER READING (mm Hg) ";S
270 IF S < -200 OR S > 400 THEN PRINT G$: PRINT "LIMITS ARE -200 TO 400": GOTO 260
280 PRINT
290 INPUT "ENTER OXYGEN CONCENTRATION (mg/L) ";Q
300 IF Q < 1 OR Q > 16 THEN PRINT G$: PRINT "OXYGEN LIMITS ARE 1 TO 16": GOTO 290
310 PRINT
320 INPUT "ENTER SALINITY (PPT)............. ";S1
330 IF S1 < 0 OR S1 > 40 THEN PRINT G$: PRINT "SALINITY LIMITS ARE 0 TO 40": GOTO 320
340 REM .....BUNSEN COEFF. BASED ON WEISS, 1970 (DEEP-SEA RES. 17:721-735)
350 T1 = T + 273.15
360 B1 = - 58.3877 + 85.8079 * (100 / T1) + 23.8439 * LOG (T1 / 100)
370 B2 = S1 * ( - 0.034892 + 0.015568 * (T1 / 100) - 0.0019387 * (T1 / 100) ^ 2)
380 B = EXP (B1 + B2)
390 REM .....VAPOR PRESS. BASED ON WEISS & PRICE, 1980 (MARINE CHEM. 8:347-359)
400 V1 = 24.4543 - 67.4509 * (100 / T1) - 4.8489 * LOG (T1 / 100) - 0.000544 * S1
410 V = EXP (V1) * 760
420 REM .....CALCULATIONS BASED ON BOUCK, 1982 (TAFS 111:505-516)
430 E = P + S
440 J = E / P * 100
450 F = Q * 0.5318 / B
460 G = F * 100 / ((P - V) * 0.20946)
470 H = (E - F - V) * 0.9877
480 I = H * 100 / (0.78084 * (P - V))
490 K = G / I
500 REM .....CALCULATIONS BASED ON COLT, 1983 (WATER RESEARCH 17:841-849)
510 H1 = E - F - V
520 H2 = H1 - 0.7905 * (P - V)
530 F2 = F - 0.20946 * (P - V)
540 GOSUB 2000
550 PRINT "WOULD YOU LIKE RESULTS PRINTED?  (Y/N)"
```

```
560 GET Q$:  PRINT Q$
570 IF Q$ = "Y" THEN 600
580 IF Q$ = "N" THEN PRINT : GOTO 770
590 PRINT G$:  GOTO 550
600 PRINT
610 REM .....CHANGE NUMBER IN STATEMENT 620 IF YOUR PRINTER IS NOT IN SLOT #1
620 PRINT CHR$ (4);"PR#1"
630 GOSUB 1000
640 PRINT "SITE= ";S$
650 PRINT "DATE= ";D$
660 PRINT
670 PRINT "INPUT DATA"
680 PRINT "----------"
690 PRINT "BAROMETRIC PRESSURE (mm Hg)=..... ";P
700 PRINT "WATER TEMPERATURE (C.)=.......... ";T
710 PRINT "SATUROMETER READING (mm Hg)=..... ";S
720 PRINT "OXYGEN CONCENTRATION (mg/L)=..... ";Q
730 PRINT "SALINITY (PPT)=.................. ";S1
740 GOSUB 2000
750 PRINT
760 PRINT CHR$ (4);"PR#0"
770 REM .....CHOICE OF ANOTHER SATURATION CALCULATION OR PROGRAM EXIT
780 PRINT "DO YOU WANT MORE CALCULATIONS?  (Y/N)"
790 GET Z$:  PRINT Z$
800 IF Z$ = "Y" THEN 120
810 IF Z$ = "N" THEN 830
820 PRINT G$:  GOTO 780
830 HOME
840 FLASH
850 HTAB 12: VTAB 12: PRINT "HAVE A NICE DAY!!"
860 NORMAL
870 END
1000 PRINT "***************************************"
1010 PRINT "*       SATUROMETER CALCULATIONS      *"
1020 PRINT "***************************************"
1030 PRINT : RETURN
2000 REM .....OUTPUT OF CALCULATED AND ROUNDED VALUES
2010 PRINT
2020 PRINT "RESULTS"
2030 PRINT "-------"
2040 PRINT "BUNSEN SOLUBILITY COEFFICIENT=... "; INT (B * 10000 + 0.5) / 10000
2050 PRINT "WATER VAPOR PRESSURE (mm Hg)=.... "; INT (V * 100 + 0.5) / 100
2060 PRINT
2070 PRINT "TOTAL DISS. GAS PRESS. (mm Hg)=.. "; INT (E * 10 + 0.5) / 10
2080 PRINT "OXYGEN PRESSURE (mm Hg)=......... "; INT (F * 10 + 0.5) / 10
2090 PRINT "NITROGEN PRESSURE (mm Hg)=....... "; INT (H * 10 + 0.5) / 10
2100 PRINT "N2 + ARGON PRESS. (mm Hg)=....... "; INT (H1 * 10 + 0.5) / 10
2110 PRINT
2120 PRINT "TOTAL HYPERB. PRESS. (mm Hg)=.... ";S
2130 PRINT "OXYGEN HYPERB. PRESS. (mm Hg)=... "; INT (F2 * 10 + 0.5) / 10
2140 PRINT "N2 + Ar HYPERB. PRESS. (mm Hg)=.. "; INT (H2 * 10 + 0.5) / 10
2150 PRINT
2160 PRINT "TOTAL PERCENT SATURATION=........ "; INT (J * 10 + 0.5) / 10
2170 PRINT "OXYGEN PERCENT SATURATION=....... "; INT (G * 10 + 0.5) / 10
2180 PRINT "NITROGEN PERCENT SATURATION=..... "; INT (I * 10 + 0.5) / 10
2190 PRINT
2200 PRINT "RATIO OF OXYGEN % / NITROGEN %=.. "; INT (K * 1000 + 0.5) / 1000
2210 PRINT "**************************************"
2220 PRINT : RETURN
```

Appendix B: Program modifications required for IBM PC.

```
120 CLS
560 INPUT Q$
610 LPRINT "************************************"
620 LPRINT "*     SATUROMETER CALCULATIONS     *"
630 LPRINT "************************************"
640 LPRINT "SITE= ";S$
650 LPRINT "DATE= ";D$
660 LPRINT
670 LPRINT "INPUT DATA"
680 LPkINT "----------"
690 LPRINT "BAROMETRIC PRESSURE (mm Hg)=..... ";P
700 LPRINT "WATER TEMPERATURE (C.)=.......... ";T
710 LPRINT "SATUROMETER READING (mm Hg)=..... ";S
720 LPRINT "OXYGEN CONCENTRATION (mg/L)=..... ";Q
730 LPRINT "SALINITY (PPT)=.................. ";S1
740 GOSUB 3000
750 LPRINT
760  (DELETE THIS LINE)
790 INPUT Z$
830 CLS
840 FOR I = 1 TO 11
850 PRINT
86´ NEXT I
870 PRINT "                         HAVE A NICE DAY!!!"
880 END
3000  REM .....OUTPUT OF CALCULATED AND ROUNDED VALUES TO PRINTER
3010  LPRINT
3020  LPRINT "RESULTS"
3030  LPRINT "-------"
3040  LPRINT USING "BUNSEN SOLUBILITY COEFFICIENT=**#.####";B
3050  LPRINT USING "WATER VAPOR PRESSURE (mm Hg)=**####.##";V
3060  LPRINT
3070  LPRINT USING "TOTAL DISS. GAS PRESS. (mm Hg)=**###.#";E
3080  LPRINT USING "OXYGEN PRESSURE (mm Hg)=**###########.#";F
3090  LPRINT USING "NITROGEN PRESSURE (mm Hg)=**#########.#";H
3100  LPRINT USING "N2 + ARGON PRESS. (mm Hg)=**#########.#";H1
3110  LPRINT
3120  LPRINT "TOTAL HYPERB. PRESS. (mm Hg)=******";S
3130  LPRINT USING "OXYGEN HYPERB. PRESS. (mm Hg)=**####.#";F2
3140  LPRINT USING "N2 + Ar HYPERB. PRESS. (mm Hg)=**###.#";H2
3150  LPRINT
3160  LPRINT USING "TOTAL PERCENT SATURATION=**##########.#";J
3170  LPRINT USING "OXYGEN PERCENT SATURATION=**#########.#";G
3180  LPRINT USING "NITROGEN PERCENT SATURATION=**#######.#";I
3190  LPRINT
3200  LPRINT USING "RATIO OF OXYGEN % / NITROGEN %=**#.###";K
3210  LPRINT "*********************************"
3320  LPRINT  : RETURN
```

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A Solid Matrix Bioassay for Determining Chemical Feeding Stimulants

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Abstract.—Attempts to manipulate the chemical composition of fish feeds and baits to increase their efficacy have required experimental studies to determine feeding preferences for species of interest. This article describes a generally useful assay method in which test stimuli are incorporated into a solid agar matrix. Agar disks are then presented to fish in a two-choice feeding assay. The agar disk assay is simple and inexpensive, requires only small quantities of test materials, and gives direct information about consumption and preference.

Attempts to manipulate the chemical composition of fish feeds and baits to increase their efficacy have required studies to determine feeding preferences for species of interest. The general approach has been to present chemical components of prey items or pure compounds to the test fish. Several methods of presentation and quantifying feeding preferences have been developed. All of these have limitations that we believe have been overcome by our bioassay procedure.

Carr and his co-workers (Carr 1976; Carr and Chaney 1976; Carr et al. 1976, 1977) developed a quantitative bioassay that involves introducing the stimuli, dissolved in sea water, through a perforated rubber striker bulb and recording the number of times a group of fish strike the bulb. While this procedure has been very useful, it does have some limitations. Because the stimuli were presented in liquid form through a rubber striker bulb, only a measure of feeding attack could be obtained rather than actual food consumption. Additionally, the delivery of test stimuli in liquid form throughout the test period requires that considerable amounts of test material be available. In studies in which natural products are being chemically isolated only small quantities may be available for testing. The striker bulb procedure makes use of automatic recording equipment and, if large numbers of test groups are to be studied, the cost of this electronic equipment can be considerable.

Delivery of test stimuli in a solid matrix has been used by Hidaka et al. (1978) and Ohsugi et al. (1978). Test substances were incorporated into starch pellets and the acceptability of the pellets by individual fish was graded visually. This procedure conserves test substances but measures intensity of feeding response directed at the pellet rather than actual consumption. This procedure is also very labor-intensive because individual fish must be continuously observed. In addition, the preparation and purification of the starch pellets is a very complex and time-consuming operation.

A third approach to examining feeding stimuli is to record swimming activity (Pawson 1977; Holland 1978; Tsushima and Ina 1978; Atema et al. 1980). Some studies have measured directed movements (e.g., entry into separate compartments) while others have only recorded swimming speed and patterns. No measure of consumption is possible because most trials involve presentation of liquid stimuli. This method also suffers from the requirement of large amounts of test material.

The standard method of our bioassay is to present test compounds to fish in a disk-shaped agar matrix. The agar itself elicits minimal feeding activity and what little consumption occurs appears to be exploratory—the fish take a few small bites when investigating the presented sample. Bioassay samples are prepared in the following way. Two percent agar (Difco, Noble) is brought to a boil and dissolved in deionized water. The solution is then allowed to cool. The appropriate quantity of test material is then added at any temperature above the solidification point (about 40°C). The agar sample solution is cast in petri dishes in which 2.5-cm-long plastic rods (e.g., computer printout binding posts) are suspended. After the agar cools and solidifies, a circular cutter (plastic tube, 1.5 cm inside diameter) is used to punch out disks of agar that have the plastic rods protruding from them. The plastic rods are attached to a mounting bracket and this assembly is placed in the test tank (Figure 1). Each mounting bracket holds an experimental and a reference disk in the two-choice preference tests. Each disk is weighed before and after a 2-h test period during which the disks are placed in a tank with a single fish. The weight

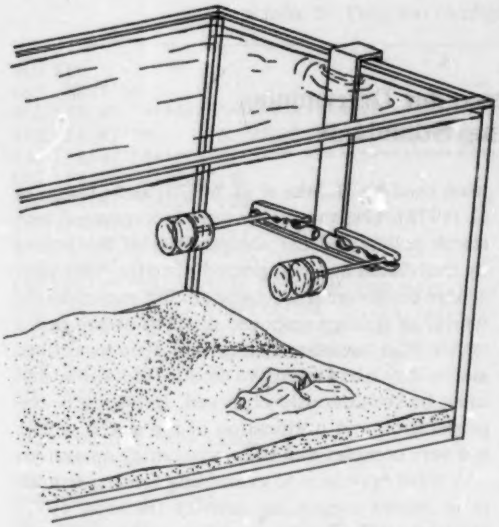

FIGURE 1.—Schematic of the agar disk bioassay test apparatus. Two agar disks are shown attached to a mounting bracket suspended in an aquarium.

differences are used to calculate consumption and preference. A set of control disks is also placed in water for the 2-h test period to measure water gain by the test disks. The agar disks typically increase in weight by 1–2% during the 2-h test period. This hydration factor is then used to calculate a corrected initial weight.

The agar disk bioassay has several advantages over previously used methods. Actual consumption is measured rather than the intensity of feeding activity or arousal. The agar itself is relatively nonstimulatory, permitting baseline consumption to be easily determined. Agar is commercially available in purified form and, because test materials are presented in a solid matrix, only small quantities are required. Table 1 presents sample data collected from redbelly tilapia (*Tilapia zilli*) by our procedure. The results of test 1, in which plain agar (the reference) was paired with agar containing 0.01 M glutamic acid (an experimental stimulus), demonstrate that the fish prefer the sample containing glutamic acid, indicating that sufficient material is released from the matrix during the 2-h test to elicit feeding. Test 2, 0.01 M glutamic acid versus 0.01 M glutamic acid, indicates that the fish exhibited no position preference in the two-choice test—both disks were sampled equally. Fish are able to detect and respond to differences in the concentration of amino acid released from the agar matrix, as shown by test 3, 0.1 M glutamic acid versus 0.01 M glutamic acid. Test 4, 0.01 M alanine versus agar alone, shows that another amino acid is an adequate feeding stimulus, while test 5, 0.01 M glutamic acid versus 0.01 M alanine, demonstrates that glutamic acid is preferred over alanine. These tests demonstrate the results that may be obtained from agar disk bioassays.

We conclude that the agar disk bioassay is simple and inexpensive to set up, requires only small quantities of test material, and gives direct information about consumption and preference.

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TABLE 1.—Results of selected bioassays with the agar disk bioassay technique. Asterisk indicates a significant preference for the experimental disk (Student's *t*-test; $P < 0.01$).

Test	Experimental disk	Reference disk	Number of trials	Mean preference for experimental disk (%±SD)
1	0.01-M glutamic acid	Blank	14	76±13*
2	0.01-M glutamic acid	0.01-M glutamic acid	15	54±15
3	0.1-M glutamic acid	0.01-M glutamic acid	16	84±19*
4	0.01-M alanine	Blank	11	65±13*
5	0.01-M glutamic acid	0.01-M alanine	14	72±17*

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Reproductive Viability of Sauger-Walleye Hybrids

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Abstract.—Artificial matings of female sauger (*Stizostedion canadense*) × male walleyes (*S. vitreum*) produced viable eggs and the resulting fry survived when stocked in a lake. Crosses of the F_1 hybrids and backcrosses of the F_1 hybrid females × sauger males also produced viable eggs and fry.

While netting walleyes (*Stizostedion vitreum*) in the headwaters of Cumberland Lake, Kentucky, during the spring of 1979, five females were captured that externally resembled walleye–sauger (*S. canadense*) hybrids. The water temperature was 52°F and walleye spawning had been in progress for several days. The suspected hybrids, along with walleye males and females, were retained in holding nets for 2 d and then transported to Kentucky's Minor E. Clark Hatchery. At the hatchery, two of the suspected hybrid females were manually spawned and the eggs fertilized with sperm from walleye males. The remaining three females were retained in indoor tanks containing water heated from 52°F to 55°F; they were injected intraperitoneally twice at a 4-d interval with human chorionic gonadotropin (HCG) and manually crossed with walleye males within 7 d. The eggs (321,000) produced from the five females were incubated at a mean water temperature of 51°F and completed hatching in 21 d. Ninety percent of the fry survived to the swim-up stage and were stocked into Cumberland Lake.

Because the five females were not positively identified as sauger–walleye hybrids, a literature search was conducted to ascertain the hybrid's reproductive capabilities. Although descriptions of the artificial hybridization of sauger and walleye (Nelson et al. 1965) and of the external characteristics of sauger–walleye hybrids (Nelson 1968) were found in the literature, information concerning the hybrid's ability to produce viable eggs or sperm was not found. An attempt was initiated to produce sauger–walleye hybrids and to raise several of them at the hatchery until maturation to determine their reproductive potential.

A sauger ♀ × walleye ♂ cross was chosen because only walleye males and females, sauger females, and the suspected hybrid females were collected in nets during the 1979 spawning run. The fish were transported to the hatchery after holding them in nets at streamside for 2 d. The male walleyes, which had free flowing milt when captured, were held in indoor tanks containing 46°F water and were not injected with HCG. The sauger females were retained in indoor tanks containing water heated to 52°F (the stream temperature at the time of capture) and were injected intraperitoneally two or three times at 4-d intervals with HCG at 200–300 International Units (I.U.) per pound of body weight. The females were spawned 10 or 11 d after the initial hormone injection and the eggs were incubated at a mean temperature of 49.5°F. The fry began hatching in 14 d and the swim-up fry were stocked 4 d after hatching was completed. Survival from egg to swim-up fry was 39%. A small number of swim-up fry were transferred to a 0.1-acre hatchery pond containing zooplankton and the fingerlings were later moved to a 1-acre pond containing forage fish. These F_1 hybrids were transferred annually to different ponds during the 4 years they were held at the hatchery; only 11 fish were retained after the first year because of a lack of forage fish.

At age III, the 10 remaining F_1 hybrids were harvested from the forage pond during the last week of March 1982. Seven of the hybrids were immature and were returned to a hatchery pond. Three of the harvested fish were females, averaging 3.2 lb in weight and 19 in in length, with fully developed ovaries. They were held in indoor tanks and injected twice at a 2-d interval with HCG (300 I.U./lb). The females ovulated within 6 d. Two of them spawned in the tank overnight. An estimated 20 oz of eggs were spawned from the remaining female and were fertilized with sauger milt. Although the egg and milt quality was poor, 4,500 fry survived to the swim-up stage and they were stocked into a small lake. The mean water temperature during the 20-d incubation period was 51.6°F. The spawned females were released into the hatchery pond containing the other seven hybrids.

Only seven F_1 hybrids (age IV) were harvested from a forage pond during March of 1983. During the 12 d prior to harvest, the pond water temperature averaged 47°F and had decreased from 52°F to 46°F. Five fish were females, averaging 3.8 lb, with fully developed ovaries; one fish was a 2.5-lb male with free flowing milt; and one was a 1.7-lb immature fish. All F_1 hybrids were retained in indoor tanks containing water heated to 52°F. The females were injected once, or twice at 4-d intervals, with HCG (200–300 I.U./lb). Ovulation occurred within 7 d.

Two of the F_1 hybrid females were crossed with sauger males, producing 142,867 eggs and 52,250 swim-up fry (36% survival). The sauger males had previously been used to fertilize sauger eggs and they were injected with HCG to increase milt production. The incubation period lasted 19 d with a mean water temperature of 54°F. The numbers of eggs per ounce, determined by counting the eggs in a 0.1-fluidounce (fl oz) sample and in a 6-in Von Bayer's trough were 3,096 and 4,460, respectively. The 0.1-fl oz egg sample was measured directly in a 0.34-fl oz graduated cylinder containing enough water to cover the eggs. The number of fry was estimated primarily by the comparison method (Piper et al. 1982).

Three of the F_1 hybrid females were spawned and the eggs fertilized with sperm from the F_1 hybrid male, producing 225,716 eggs and 100,000 swim-up fry (45% survival). The mean water temperature during the 18-d incubation period was 54.5°F. The numbers of eggs per ounce, estimated volumetrically and by Von Bayer's method were 3,315 and 4,822, respectively. The number of fry was again estimated by the comparison method.

Two 0.1-acre hatchery ponds containing zooplankton were separately stocked with 5,000 to 5,700 swim-up fry from one of the two crosses. The ponds were drained after 40 d; the harvested fingerlings ranged in length from 1.3 to 1.4 in. The survival rate from swim-up to fingerling stage was over 90% for both crosses.

The F_2 and backcrossed fingerlings were stocked separately into several small lakes. The post-stocking survival of these fish has not been determined, but F_2 fish have been caught by anglers 16 months after release.

In conclusion, our experiments have shown that F_1 hybrids produced by crossing sauger females with walleye males are capable of producing viable eggs and milt and can be successfully backcrossed with sauger. The backcrossed and F_2 fry and fingerlings produced during this study exhibited normal development and survival for at least 40 d; F_2 fish have been observed 16 months after stocking. Although our work was conducted under hatchery conditions, it appears that this hybrid may be capable of spawning naturally.

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Waste Solids Control in Hatchery Raceways

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Abstract.—Concerns over water pollution from fish hatcheries have resulted in the design of various systems to control suspended solids in hatchery discharges. The State of Michigan has incorporated baffles in its hatchery raceways to move the solids to the foot of the raceways where they can be removed. This has eliminated the need for brushing raceways and for treating the large volume of water that resulted from this activity. All cold water species (*Salvelinus namaycush*), except lake trout, have adapted well to baffles in raceways.

The control of solid waste products in intensive fish production confronts all hatchery managers. Studies of hatchery operations (Dydek 1972; Michigan Water Resources Commission 1973) showed that the major contribution of solids and phosphorus to the receiving waters occurs during raceway cleaning. Although various systems were designed to treat the cleaning flows (Great Lakes Fishery Commission 1972), time consuming raceway cleaning, with its associated stress on fish, was still necessary. Raceway cleaning also was a problem for hatcheries designed with serial reuse. Either the solids needed to be moved from raceway to raceway or flows to lower ponds in the series had to be temporarily reduced or even interrupted. To solve these problems, baffles were placed in a raceway as a mechanism for self-cleaning. Additionally, they would allow the fish to select varying water velocities. This paper outlines Michigan's experiences with solids control with baffled raceway systems.

A generalized schematic of Michigan's hatchery design is presented in Figure 1. The baffled raceway system is designed to move solid waste material to the end of the raceway (Figure 2). The solids continuously move along the length of the raceway and deposit in a special raceway section, behind a fish retaining screen, from which they can be removed. The distance between the baffles should be about equal to the width of the rearing unit.

The space between the bottom edge of the baffle and the floor of the raceway must be small enough to create sufficient velocity to move the solids down the raceway. Velocities that are too high increase shear stresses which can cause solids to break up, reducing their settling rates. Burrows and Chenoweth (1955) found that 0.24–0.3 m/s was the minimum velocity needed to prevent the settling

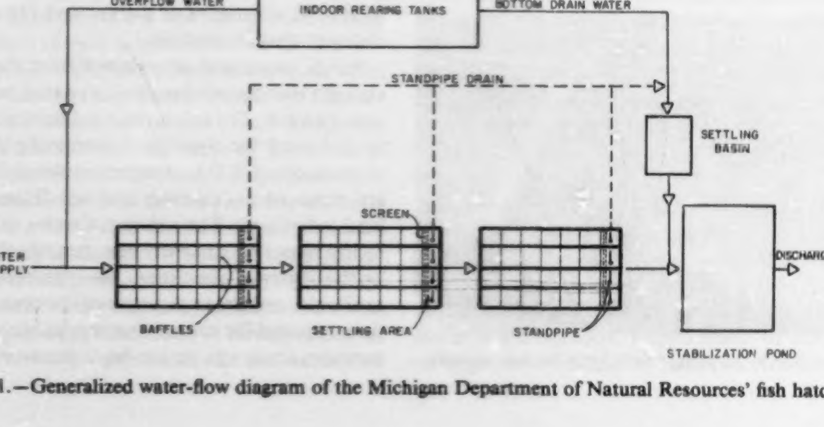

FIGURE 1.—Generalized water-flow diagram of the Michigan Department of Natural Resources' fish hatcheries.

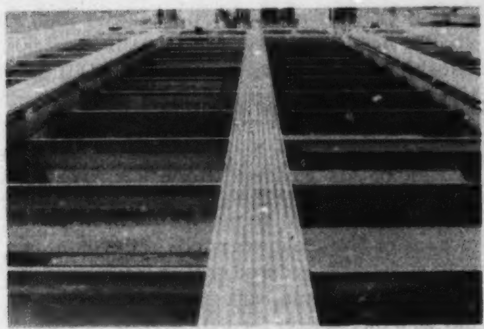

FIGURE 2.—Baffles in a raceway that is 30 m long and 3 m wide and has an operating depth of 0.67 m.

FIGURE 4.—Removing solids to a hauling truck by means of a gas powered pump.

of salmon waste. The velocity needed immediately under the baffle can range from 0.1 to 0.4 m/s, depending on fish size and species and the characteristics of the rearing unit. The velocity under the baffle can be calculated from the equation $Q = VA$ (Streeter 1971), where Q is flow (m^3/s), V is velocity (m/s), and A is area perpendicular to the flow (m^2). Because Q is the same both upstream and at the baffle and because the width also remains constant, the velocity can be calculated as

$$V_b = V_a\left(\frac{D_1}{D_2}\right)$$

where V_b is the velocity under the baffle, V_a is the velocity upstream of the baffle, D_1 is the depth of the water in the raceway, and D_2 is the depth under the baffle.

The space provided at the end of the raceway for solids collection is sized about the same as the distance between baffles. In a settling area of 3 m square, with a 0.67-m operating depth, and maximum design velocity of 0.033 m/s, all particles with a settling velocity greater than 0.0074 m/s will, theoretically, settle to the bottom. The settling velocity of fecal matter from fish of a size normally found in outdoor raceways (Alabaster 1982) is greater than this velocity. Food particles, after water absorption, will also settle fast enough to stay in the settling area.

FIGURE 3.—Vacuuming solids from the settling area.

The baffles were constructed of 1.59-mm aluminum because they are not required to sustain much pressure. Generally, the baffles can be held in place with one of two techniques: (1) slots in the sides of the raceway walls; or (2) bolts on the baffles, which are placed in holes in the raceway walls and locked in place like a door bolt.

The head loss is negligible with a baffled raceway. The head loss designed into the 30-m raceway is only 0.15 m. Use of the baffles in dirt raceways does not seem practical and has not been attempted.

Solids, concentrated in the settling area, should be removed with as little water and labor as possible. Two methods have been used to remove solids: (1) a vacuum or pump; and (2) a bottom cleanout drain (standpipe).

Solids vacuumed or pumped from the settling area are transferred directly into a tank truck (Figures 3 and 4). The solids removal device is similar to that used for cleaning a swimming pool. Approximately 5,700 L, containing about 5% solids, are removed per raceway and are disposed of on land as fertilizer. The volume of water and percent solids removed can vary significantly depending on the degree of cleanliness, the type of equipment available, and the experience of the operator. The time required for vacuuming or pumping generally averages about one person-hour per raceway. This includes time required for equipment preparation, cleanup, solids disposal (within 1.5 km of hatchery), and work breaks and requires a two-person cleaning team.

Bottom drain solids removal involves placing an aluminum barrier just upstream of the settling area and removing a standpipe from the center of the settling area; the solids are flushed into a settling basin. The volume of water required for flushing is 6,100 L. Clarified water is routed to a stabilization pond, as is all other raceway water. Solids removal by this method can be done by one individual in 30 min.

Special problems can occur with the standpipe system. Some are caused by the placement of the barrier in the raceway. If water is discharged into the raceway when the barrier is in place, water will back up in the raceway. Also, while the barrier is in place no water exchange will occur. Other problems involve the settling basin. If sufficient head is not available to decant water out of the settling basin into the stabilization pond between raceway cleanings, the discharge of the raceway water into the settling basin causes an almost continuous water flow to the basin. Michigan has switched almost exclusively to removing solids by pumping because of the above problems.

Baffles increase the difficulty of removing biological growths from the raceway walls and bottom, and any permanent piping for pumping solids must be drainable to avoid freezing when not in use in the winter. Solids also tend to pile up in front of the fish retaining screen when an excess amount of solids have collected in the settling area.

The frequency of solids removal is dependent on the amount of fish food being fed. At low production levels (0.5 kg fish per L/min) solids removal can be twice a month. At higher levels (1.5–2.0 kg fish per L/min) it may be necessary to remove it every 3–4 d.

Baffles have also been used in indoor rearing tanks, which ranged in size from 0.3 to 12.0 m^3. Generally, the baffles are placed in the tanks when small fish can cope with them. Usually the number of baffles is increased in the tanks until the spacing between them is about the same as the width of the tanks. Solids are collected in a gutter just below the fish-retaining screen. Solids are removed daily by opening a valve and flushing them to a small solids-settling basin which discharges to the hatchery stabilization pond.

Suspended solids control with baffles and a stabilization pond has been found to be very effective. Discharge permit requirements for net suspended solid concentrations of 4 mg/L on a 30-d average and 6 mg/L for a daily maximum have consistently been achieved. Raceway discharges into the stabilization pond have, on some occasions, had lower suspended solids (2–4 mg/L) than the discharge from the stabilization pond (5–6 mg/L) because of algae growth in the pond.

Elevated levels of suspended solids (10–20 mg/L) have been found in the raceway discharge to the stabilization pond because of the turbulence caused in the settling area during vacuuming. It is desirable to maintain the overflow structure from the settling area at least the same width as the raceway. In raceways where V-notch weirs have been used as flow-control structures, some of the solids are discharged during normal operations because V-notch weirs cause a greater velocity in the area of notch compared to the full channel weir.

Solids control in a baffled raceway with a vacuum removal system is more labor than capital intensive. The major capital cost will be the purchase of a vehicle to haul solids. The number of raceways to be cleaned, the distance sludge must be hauled, and the conditions under which sludge is disposed must all be taken into consideration during vehicle selection. The further the distance the solids must be hauled, the larger the truck should be in order to minimize hauling time. If solids must be transported year-round, a four-wheel drive vehicle may be necessary.

A standpipe removal system requires significantly greater capital costs than a vacuum system. These costs can be offset through the life of the facility with the reduced labor and equipment costs. Labor costs are lower because one person can handle this job whereas two are required with a vacuuming system. Fewer disposal trips are also required to haul the more concentrated solids.

No adverse effects on fish have been observed with the use of baffles in outdoor raceways. The baffles are placed in the raceway immediately after the fish are moved from the indoor rearing tanks to the raceway. Baffles have not worked well with lake trout (*Salvelinus namaycush*), but they have been effective with coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout and steelhead (*Salmo gairdneri*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*). The baffles are arranged in the same manner for all species up to 1–1.5 years old. When baffles are used during the initial indoor rearing phase, they should not be put in place too soon. Brown trout, in the swim-up stage, have reacted to baffles by crowding into the downstream end of the tank. This phenomenon continues until the fish are ready for the outdoor raceways when they are about 5 cm long. Other species have adopted well to the baffles.

The use of baffles has helped reduce the labor required for raceway cleaning. Baffles also help solve the problems associated with cleaning raceways that contain fish and with treating the large volumes of water generated during raceway cleaning. More importantly, the use of baffles in raceways has proven to be very successful in reducing solids in the effluents of the Michigan Department of Natural Resources' fish hatcheries.

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Chrysaora quinquecirrha: A Predator on Mysids (*Mysidopsis bahia*) in Culture[1]

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A closed-system culture of *Mysidopsis bahia* has been maintained at the Virginia Institute of Marine Science since 1978 to provide test animals for aquatic toxicity tests. The mysids are cultured in 37-L glass aquaria that are equipped with undergravel filters and a crushed oyster shell substrate covered with 1.5-mm plastic screen. The tanks are filled with water from the York River, Virginia (salinity 15–25‰); the water is filtered at 1 μm and maintained at 25 ± 1°C. Mysids are fed 48-h-old *Artemia* nauplii daily.

In January 1984, following replacement of the crushed oyster shell substrate, a rapid decline in mysid populations was observed. *Artemia* also disappeared at higher rates than could be accounted for by the diminished numbers of mysids. Examination of the substrate revealed huge numbers of scyphopolyps feeding on both *Artemia* and juvenile mysids. The polyps ranged in size from 1 to 2.5 mm and had a mean number of 16 tentacles. Nematocysts were clustered in rings around the tentacles. The mouth occupied the entire oral region of the polyp. Polyps were identified by culturing them in the laboratory until strobilization occurred and the morphologically distinct ephyrae were present. When polyps from the mysid cultures were removed to finger bowls, strobilization was brought about by overfeeding with *Artemia*. The ephyrae lacked gastric cirri and possessed the slender pointed lappets and pale pink coloration characteristic of the sea nettle, *Chrysaora quinquecirrha* (Russell 1970; Calder 1972).

Chrysaora quinquecirrha is common in the mesopolyhaline waters of the Chesapeake Bay. Polyps have been found inhabiting the surface of oyster shells from May through mid-December (Cargo and Shultz 1966), but individuals may live for several years strobilating each summer (Littleford

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[1] Contribution 1276 from the Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point.

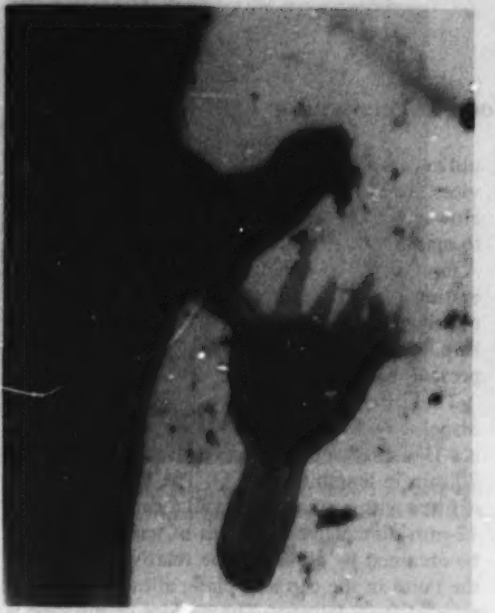

FIGURE 1.—*Chrysaora quinquecirrha* producing a somatic bud (0.3 mm long), and a small polyp (0.6 mm long) released from the same parent 3 d earlier.

1939). Under adverse conditions of low oxygen concentration or temperatures below 4°C, the polyps encyst, forming small 2-mm-diameter cysts. The appearance of *Chrysaora* contaminants in the culture tanks presumably was a result of cysts being introduced with oyster shell chips.

C. quinquecirrha polyps can have catastrophic effects on cultures of small crustaceans because of their ability to undergo rapid asexual reproduction exclusive of strobilization. Littleford (1939) stated that scyphistomae may be produced by somatic budding from the stalk of the polyp or by the formation of stolons; however, he believed both events were rare in the species. We found an abundance of somatic buds (Figure 1). Polyps, when well supplied with *Artemia*, were able to bud every 5–6 d. Buds were able to feed, while still attached to the "parent" organism, once several tentacles had developed.

We added several hermit crabs (*Pagurus longicarpus*) to one of the mysid tanks as recommended by Lawler and Shepard (1978) for control of hydrozoan pests. Within 3 d the crabs had completely cleared the surface of the substrate but they were unable to attack the polyps below the plastic screen and in the lift tubes. The tanks were disassembled; tanks and undergravel filter parts were scrubbed thoroughly and treated with a dilute (1:160) Clorox (sodium hypochlorite) solution. New crushed oyster shell substrate was autoclaved (25 min at 1.06 kg/cm^2) before the tanks were reassembled in order to kill any *Chrysaora* cysts that may have been present. Five days after we inoculated the cleaned tanks with 50 adult mysids numerous juvenile mysids were observed, and within 3 weeks we counted more than 200 mysids. No *Chrysaora* polyps reappeared during the subsequent 4-month period.

We believe Clorox is superior to formalin for disinfection of tanks and equipment. Clorox should be applied only after removal of the mysids. Formalin was used by Lawler and Shepard (1978) to treat tanks contaminated with hydrozoan polyps while mysids were present. Formalin is highly toxic, and a severe depletion of mysids occurred in their system following formalin treatment. Further, there is significant potential for persistence of formalin in the tank following treatment, whereas chlorine is rapidly reduced within the tank and can be deliberately removed by addition of thiosulfate, a nontoxic reducing agent.

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An Improved Automatic Dry Food Dispenser for Fish Larvae

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Abstract.—An automatic dispenser of dry fish food is described that effectively disperses small quantities of very fine granules of food. It is well adapted for supplying food to small groups of fish that must be fed at frequent intervals.

A major problem encountered in the rearing of young fish larvae with dry food instead of live zooplankton is ensuring an adequate supply of food particles in the water. Live prey remain available for a long time after their introduction into rearing tanks, but dry food particles tend to sink to the bottom of the tank where they lose their availability. Attempts to keep these particles in suspension with water currents have some inherent drawbacks, such as loss of nutritive value due to leaching. Better larval feeding can be accomplished by continually dispensing food particles at carefully adjusted delivery rates. Insufficient food supply leads to starvation of larvae, and excess feeding leads to deteriorated water quality from accumulation of uneaten food. Automatic feeders have been reviewed in detail by Berka (1973); these utilize apertures closed by electromagnetic devices, endless screws, disks, or moving paths. However, these feeders appear to be more adapted to mass rearing than to small-scale larval rearing. A food dispenser was designed for small-scale operations (Charlon and Bergot 1984) based on the rotating system introduced by MacFarlane (1976). Improvements in their design are described in the present work.

The feeder includes two removable units—a food dispenser and a holder. The food dispensing unit is a 33-mm-diameter, transparent polystyrene tube, 70 mm in length (Figure 1). The top of the tube is fitted with a small plate and a cap, each with a 12-mm-diameter hole. A slit of varying size can be obtained by adjusting the relative position of the holes in the cap and plate, allowing different rates of food delivery. The bottom of the tube is removed and the threaded female part of a 32-mm-diameter polyvinyl-chloride (PVC) plug is glued inside.

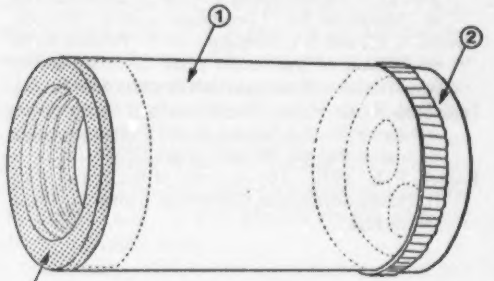

FIGURE 1.—Food dispensing unit: (1) transparent polystyrene tube; (2) cap over plate (the two respective holes are shown in dotted lines); (3) threaded female polyvinyl-chloride plug (shaded area).

FIGURE 2.—Holder unit: (1) polyvinyl-chloride (PVC) support on which the threaded male PVC plug is glued; (2) base composed of two PVC blocks attached with two screws; (3) small pin for articulation of element 1 with element 2; (4) main rotation shaft.

FIGURE 3.—The assembled feeder shown in operation (near the equilibrium position); the main shaft rotates toward the viewer.

The holder unit includes two parts hinged around a small, 1.5-mm, stainless-steel pin (Figure 2).

(1) A PVC support is glued to the threaded male part of the PVC plug. The food dispensing unit is screwed on this support by means of the male and female parts of the PVC plug (Figure 3).

(2) A base is composed of two PVC blocks—50 × 35 × 10 mm and 35 × 30 × 10 mm. An 11.5-mm-diameter hole is drilled through these two blocks in which a 12-mm-diameter stainless-steel shaft can be fixed; this shaft is used as the rotation axis for the feeder. The blocks are held together around the shaft with two 20 × 3-mm screws.

The rotation shaft is driven by a small rotisserie-type synchronous-motor gearbox (24 V AC, one revolution per minute) attached to one end. The connection between motor and rotation shafts is accomplished with a 6–12-mm reducing sleeve. The electric motor is regulated by a cam programmer with a 10-min cycle. The other end of the rotation shaft is held by a ball bearing.

The feeding equipment in our experimental hatchery is shown in Figure 4. Two series of 10 food dispensers supply food to two rows of 10 3-L tanks. The system includes two 3-m-long shafts driven by two synchronous motors connected to a 2-cam programmer. Each shaft is held by two ball bearings and supports 10 feeders. This system allows testing of various diet formulations and particle sizes at a programmed delivery rate.

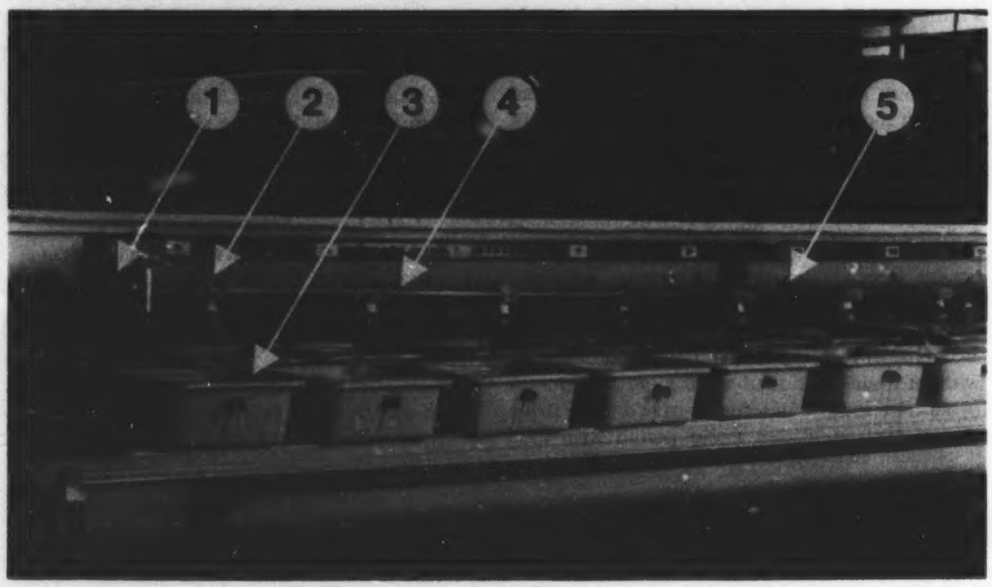

FIGURE 4.—Operational configuration of food dispensers in our experimental hatchery: (1) rotisserie-type motor gearbox (the cam programmer is located in the electric control box); (2) a food dispenser; (3) fish rearing tank; (4) main rotation shaft; (5) ball bearing.

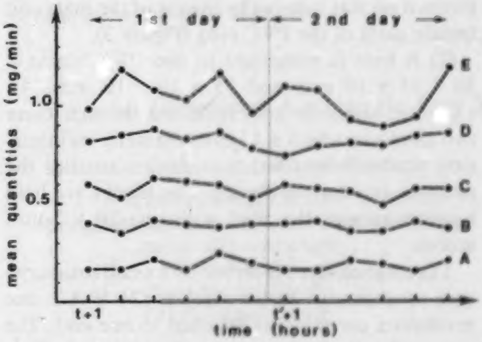

FIGURE 5.—Mean quantities of food delivered at each rotation of the feeder averaged over 1 h. Particle sizes: A, 100–200 μm; B, 200–400 μm; C, 400–630 μm; D, 630–800 μm; E, 800–1,250 μm. The first hours after the start of the food delivery on days 1 and 2 are marked by t + 1 and t' + 1, respectively.

At each rotation of the main shaft, the dispensing unit is raised and then falls and strikes strongly against the base (Figure 3). During this rotation, the food particles move about inside the dispensing unit, which prevents them from aggregating. At each strike, a limited quantity of food particles is projected through the adjustable slit. The dispensing unit is very easily unscrewed for refilling. Filling must always be done with plate and cap in the lower position and a strip of adhesive tape covering the slit to prevent food particles from falling out. The dispensing unit can be unscrewed to weigh the remaining food; thus the amount of food distributed can be calculated without changing the distribution slit.

Delivery performances were investigated for the 100–200-, 200–400-, and 400–630-μm particle sizes of experimental diets used for carp and coregonid larvae. Commercial trout starter food, measuring 630–800 or 800–1,250 μm, was also tested. The width of the slit should be adjusted for each type of food particle. Trials were performed with an initial food load of 20 g per unit during two 6-h periods on two consecutive days; a strike rate of 60/h simulated a 2-d period with four strikes every 10 min for 15 h/d. Food was collected hourly and weighed to the nearest 0.01 g; mean quantities delivered at each rotation of the feeder were calculated (Figure 5). Coefficients of variation were about 5–15%. Differences in total delivered quantities between the first and second days were less than 4%.

TABLE 1.—Minimal and maximal quantities (mg) of food that can be delivered at each rotation of the feeder.

Particle size (μm)	Quantities (mg)	
	Minimal	Maximal
100–200	0.8	33
200–400	1	50
400–630	1.7	50
630–800	1.7	50
800–1,250	3	67

There are evident limits to the slit-width adjustment. Too small an aperture will be obstructed, whereas too large an aperture will allow food to flow out. Minimum and maximum delivery rates are given in Table 1. Another limitation is the irregularity of delivery that occurs when the food level is too low in the feeder (i.e., less than 6 g of 100–200-μm starter diet). In spite of these limitations, the feeder is quite versatile and has a large range of delivery rates.

More than 30 feeders have been in regular use in our experimental hatchery over 2 years without problem; they are easily dismantled and cleaned and all components are corrosion-resistant. For larger tanks (e.g., 1,500 L) the same feeders are used in series on the same shaft. The additional feeding points ensure a better distribution of food particles.

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ERRATA

Please make the following changes in a recent issue of *The Progressive Fish-Culturist.*

Volume 47(4), October 1985: "A Self-Supporting, Inexpensive Gravity Aerator," by C. T. Fridirici and L. T. Beck, pages 248–250.

Interchange the formula for gravity aeration efficiency given on page 249 with the formula for dissolved gas levels given on page 250.

Volume 47(4), October 1985: "A Pilot-Scale Reuse System for Salmonids," by S. G. Hughes and R. C. Williams, pages 251–253.

In the caption for Figure 1 on page 251, add the underscored phrase: ". . . the remaining flow goes through a head tank (E) and on through ozone columns (F) to either hatchery jars (G) or troughs (H)."

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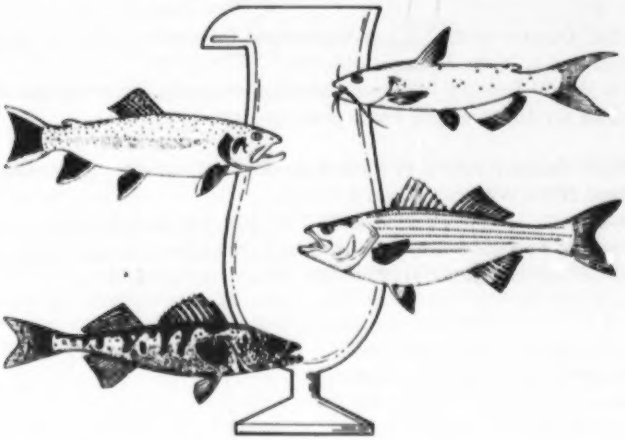

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Prefixes

kilo (10^3)	k
milli (10^{-3})	m
micro (10^{-6})	μ

Time and Temperature

day	d
degrees Celsius ([°F − 32]/1.8)	°C
degrees Fahrenheit ([1.8 × °C] + 32)	°F
hour (spell out for diel time)	h
minute	min
second	s

Spell out year, month, and week.

Weights and Measures (Metric)

centimeter (0.394 in)	cm
gram (0.0353 oz)	g
kilogram (2.20 lb)	kg
kilometer (0.622 mi)	km
liter (0.264 gal, 1.06 qt) (exception to SI)	L
meter (1.09 yd, 3.28 ft, 39.4 in)	m

Weights and Measures (English)

cubic feet per second (0.0283 m^3/s)	ft^3/s
foot (30.5 cm)	ft
gallon (3.79 L)	gal
inch (2.54 cm)	in
mile (1.61 km)	mi
ounce (28.4 g)	oz
pound (0.454 kg, 454 g)	lb
quart (0.946 L)	qt
yard (0.914 m, 91.4 cm)	yd

Spell out acre (0.405 hectare), hectare (2.47 acres), ton (2,000 lb, 907 kg), and tonne (1,000 kg, 2,200 lb).

Mathematics and Statistics

all standard mathematical signs, symbols, and abbreviations	
base of natural logarithm	*e*
common test statistics (*F*, *t*, etc.)	
correlation coefficient (multiple)	*R*
correlation coefficient (simple)	*r*
degree (angular or temperature)	°
degrees of freedom	df
logarithm (specify base)	log
minute (angular)	′
not significant	NS
percent	%
probability	*P*
sample size	*N*
second (angular)	″
standard deviation	SD
standard error	SE

Physics and Chemistry

all atomic symbols	
alternating current	AC
ampere	A
British thermal unit (1.05 J)	Btu
chemical acronyms listed in Webster's dictionaries (DDT, EDTA, etc.)	
direct current	DC
footcandle (0.0929 lx)	fc
hertz	Hz
hydrogen ion activity (negative log of)	pH
joule (0.239 gram-calories, 0.000948 Btu)	J
lux (10.8 fc)	lx
molar	M
mole	mol
ohm	Ω
ortho	*o*
para	*p*
pascal	Pa
parts per million (per 10^6) (in the metric system, use mg/L, mg/kg, etc.)	ppm
parts per thousand (per 10^3)	ppt, ‰
siemens (=mho, Ω^{-1})	S
volt	V
watt	W

Calories should be converted to joules in the metric system.

General (Some Are Restricted)

compass directions (maps and coordinates): east	E
north	N
south	S
west	W
District of Columbia	D.C.
et alii	et al.
et cetera	etc.
for example	e.g.,
months (tables, figures): first three letters (Feb, Jun, etc.)	
sex (tables, figures, hybrid crosses): female	♀
male	♂
that is	i.e.,
Union of Soviet Socialist Republics	USSR
United States (adjective)	U.S.
United States of America (noun)	USA

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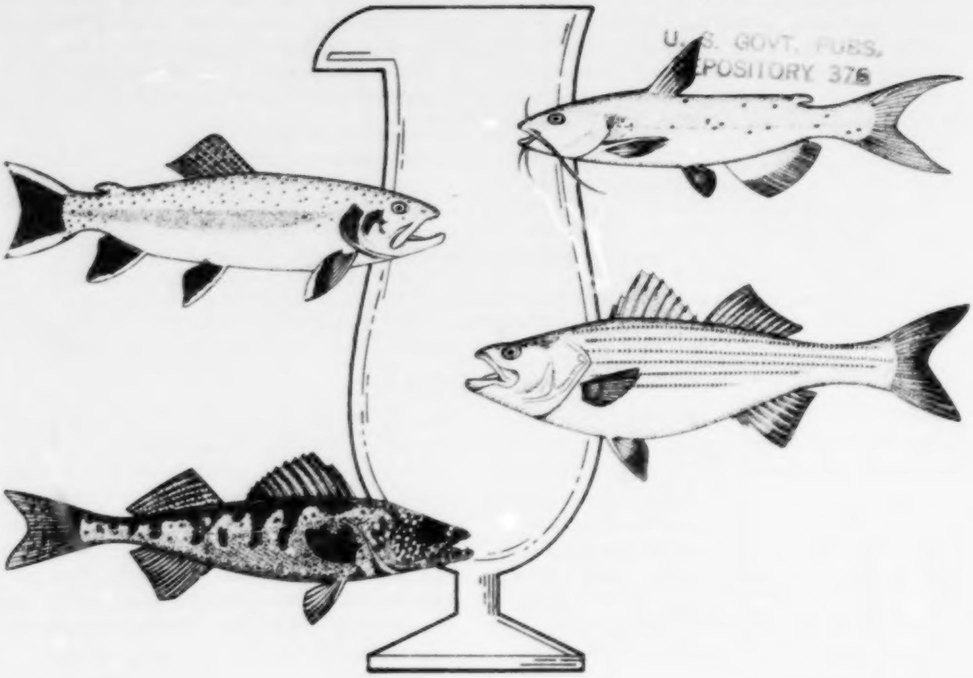

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Tilapia Tolerance of Saline Waters: A Review

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Abstract.—Members of the family Cichlidae that are generally referred to as tilapias (*Tilapia* spp.) characteristically demonstrate some degree of euryhalinity. A survey of existing literature indicates that *Tilapia zilli* and *Tilapia mossambica* are among the most salinity-tolerant species, although neither is among the most desirable for culture. A tolerance for high salinity has been demonstrated in at least some crosses that produce red hybrid tilapia. Most known heritage crosses with that result to date have involved *T. mossambica.* The red hybrid may become a preferred culture fish in brackish and seawater systems as well as in fresh water.

Many species of tilapia (*Tilapia* spp.) are broadly euryhaline (Chervinski 1961a), while others are restricted to fresh or low-salinity waters. It has been speculated that tilapias evolved from a marine ancestor and secondarily invaded freshwater environments (Myers 1938; Steinitz 1954). Various species of *Tilapia*, most of which have not been cultured, occur in African lakes; others occur in Middle Eastern lakes where salinities may reach or exceed that of seawater during seasonal drought periods. Reports on tilapia species of little or no aquacultural interest from lakes, estuaries, and coastal areas of Africa include those of Mortimer (1962), Coe (1966, 1967, 1969), Morgan and Kalk (1970), Fagade (1971), Fryer and Iles (1972), Clarke (1973a), Beadle (1974), Reite et al. (1974), Pauly (1976), Whitfield and Blaber (1976), Philippart and Ruwet (1982), and Payne (1983). *Tilapia galilaea*, found in Israel as well as in Egypt, grows and reproduces over a relatively wide salinity range (El-Zarka et al. 1970; Kirk 1972). These fish can survive direct transfer from fresh water to 19.5‰ salinity (Chervinski 1961c), but generally the species is of little value to aquaculture (Sarig 1955).

Prior to the development of various techniques to control overpopulation and consequent stunting of cultured tilapias, Hickling (1963) advocated rearing the fish in brackish water as a means of limiting reproduction. He also indicated that some growth improvement could be obtained through brackish-water culture. Although no species name is provided in the paper, Hickling (1963) was most likely referring to *T. mossambica* in his discussion.

Many tilapia culturists currently prefer *T. aurea* and *T. nilotica* to *T. mossambica*. Because *T. mossambica* was the first tilapia to receive widespread distribution outside the native African and Middle Eastern range of the genus, it is still widely cultured and has often been hybridized with other introduced tilapia species. Such hybrids, as well as species misidentifications, may account for some of the apparent discrepancies among results from similar studies performed on what were reportedly the same species.

Previous reviews of tilapia salinity tolerance have been written by Fryer and Iles (1972), Balarin and Hatton (1979), Chervinski (1982), Philippart and Ruwet (1982), and Wohlforth and Hulata (1983).

This review contains citations listed by those authors plus additional references.

Studies involving tilapia in saline waters include basic research on the physiology of salinity stress in fish as well as more practical research on aquacultural practices. In both instances, relatively few species (mostly those of aquacultural interest) have dominated the literature. Species of minor aquacultural interest have largely appeared in reports of their occurrence in brackish water arising from life-history or ecological studies. An exception, *T. zilli*, has been the subject of several papers on its tolerance for, and performance in, saline waters (El-Zarka 1956; Bayoumi 1969; El-Zarka et al. 1970; Fryer and Iles 1972; Chervinski and Hering 1973; Farghaly et al. 1973; Chervinski and Zorn 1974; Leatherland et al. 1974; Balarin and Hatton 1979; Whitfield and Blaber 1979; Payne and Collinson 1983). *T. zilli* survive and grow in salinities of at least 40‰ (Chervinski and Hering 1973; Balarin and Hatton 1979) and reproduce at salinities of at least 29‰ (El-Zarka 1956; El-Zarka et al. 1970). Payne and Collinson (1983) reported reproduction at 40‰ salinity, but Chervinski and Zorn (1974) found no reproduction in waters of 38.8 to 43.7‰. A few laboratory studies on the salinity tolerance of other minor tilapia species have also been published (Chervinski 1961a; Fukusho 1969; Leatherland et al. 1974).

Species of Aquacultural Interest

Tilapia aurea and T. nilotica

Several studies have been conducted with *T. aurea, T. nilotica,* and their hybrids in relation to saline waters; most have dealt with the introduction of fish to brackish water in culture ponds or in the laboratory. Both species have been reported in brackish lakes in Egypt (Fryer and Iles 1972; Kirk 1972), and both once occurred in Lake Quarun but were displaced by *T. zilli* as the lake became more saline (Fryer and Iles 1972). Kirk (1972) reported that *T. nilotica* was recovered from the Bitter Lakes of Egypt at salinities ranging from 13 to 29‰.

Through acclimation, Coleman et al. (1977) were able to maintain *T. aurea* in two-thirds strength seawater (23‰). Fukusho (1969) reported that *T. nilotica* tolerated direct transfer to 50% seawater (17.5‰), but not 75% seawater (26‰). Lotan (1960) indicated that *T. nilotica* survived direct transfer from fresh water to 60% seawater (21‰) and could be acclimated to 150% seawater (53‰). According to Balarin and Hatton (1979), Lotan (1960) misidentified *T. aurea* as *T. nilotica.*

A limited amount of work has related temperature and salinity to performance by *T. aurea* and *T. nilotica.* Chervinski (1966) found that *T. aurea* was better able to tolerate cold temperatures in 5‰ salinity than in fresh water. Beamish (1970) determined that the preferred temperature of *T. nilotica* was 20–25°C at salinities of both 0 and 30‰.

The optimum salinity range for growth has not been well established for either *T. aurea* or *T. nilotica.* Chervinski (1966) reported that growth of *T. aurea* was nearly the same in fresh water and 10‰ salinity, although mortalities were higher in the brackish water. Good growth has been reported in salinities up to 40‰ for the same species (Chervinski and Yashouv 1971; Chervinski 1972; Chervinski and Zorn 1974), although *T. aurea* were affected by external lesions in high-salinity water (Chervinski and Zorn 1974). Payne and Collinson (1983) reported that the most effective growth for *T. aurea* occurred in brackish water of 10–15‰ salinity; best growth of *T. nilotica* occurred at 5–10‰. Hybrids between the two species have demonstrated rapid growth at salinities up to 18‰ (Fishelson and Loya 1969; Loya and Fishelson 1969). Somewhat contradictory is a study by Payne (1983) in which *T. aurea* × *T. nilotica* hybrids grew well at 6‰, but more poorly at higher salinities. Because studies of this nature have not been conducted over a broad salinity range simultaneously, it is difficult to compare them with each other.

Tilapia nilotica fry are intolerant of direct transfer from fresh water to seawater until 45 d posthatch (Watanabe 1985); tolerance increases to a maximum at 150 d posthatch. Similar results were reported in *T. aurea.*

Tilapia aurea has reproduced in salinities of 4.3‰ (Perry and Avault 1972) and 6‰ (Chervinski 1966). No reproduction occurs within the salinity range of 36.6 to 44.6‰ (Chervinski and Yashouv 1971), but the salinity at which reproduction is curtailed has not been adequately established. Reproduction by *T. nilotica* has been observed at 18.9‰ salinity (Chervinski 1961b).

Tilapia mossambica

The most widely studied species of tilapia with respect to saline waters is *T. mossambica.* It has commonly been a subject of physiological studies, most of which have employed maximum salinities no higher than 35‰ (Dharmamba and Nishioka 1968; Job 1969a, 1969b; Clarke 1971, 1972, 1973a, 1973b; Kruger 1971; Zambrano et al. 1972; Dharmamba et al. 1973; Krishnamurthy and Bern 1973; Narasimham and Parvatheswararao 1973; Venkatachari 1974; Dharmamba and Maetz 1976; Assem and Hanke 1978, 1979b; Loretz 1979; Mainoya 1982; Mainoya and Bern 1982).

Following their introduction into fresh waters around the world, *T. mossambica* have been subsequently found thriving in marine environments. Such habitat shifts have taken the fish into estuaries in Puerto Rico (Austin 1971) and Papua New Guinea (Gluckman et al. 1976) and into a brackish pond in Hawaii (Neil 1966). A breeding population has been reported from an atoll in the Pacific Ocean (Lobel 1980) where salinities doubtless are no lower than 35‰.

Tilapia mossambica does not tolerate direct transfer to full-strength seawater from fresh water (Fukusho 1969), but the fish can be readily acclimated to 35‰ salinity (Miller and Ballantine 1974) and higher. Acclimation salinities of 60, 70, and 120‰ were achieved by Assem and Hanke (1979a), Potts et al. (1967), and Whitfield and Blaber (1979), respectively. Direct transfer to 27‰ salinity appears possible without mortality (Assem and Hanke 1979b). In addition, although 5-mm *T. mossambica* cannot survive direct transfer from fresh water to seawater, they will survive the reverse transfer (Brock 1954). Maximum salinity tolerance by *T. mossambica* is reached when fish are 52 mm long (Watanabe 1985).

The salinity tolerance of hybrid tilapia fry (*T. mossambica* female × *T. nilotica* male) begins to increase 7 d posthatch. In contrast, improved salinity tolerance of *T. nilotica* or *T. aurea* occurs much later (Watanabe 1985).

Numerous reports of rearing *T. mossambica* in brackish water have been published. Some of the earliest work, attributed to *T. mossambica*, was performed by le Mare (1950) and Schuster (1952), as discussed by Hickling (1970). Good growth in brackish water was reported by both authors; le Mare (1950) reported a salinity of 20‰. Good growth has also been reported at 30‰ (Chimits 1957) and 35‰ (Uchida and King 1962; Canagaratnam 1966). Growth is reportedly better at a salinity of 17.5‰ than of 35‰ (Canagaratnam 1966); an increased metabolic rate due to osmoregulatory stress may occur at salinities higher than 17.5‰ or lower than 8.75‰ (Bashamohideen and Parvatheswararao 1972). The improved growth of *T. mossambica* in brackish water compared with growth at high and low salinities seems to be physiologically related to osmoregulatory function.

Survival in ponds has been reported at salinities as high as 42‰ (Gundermann and Popper 1977; Pinto 1982). *Tilapia mossambica* has been reared in intensive culture systems at salinities of 5 to 19‰ (Kuhlmann 1976), and for an extended period at 8‰ (Otte and Rosenthal 1979).

Spawning by *T. mossambica* has been reported at 30‰ (Vaas and Hofstede 1952; Chimits 1957; Fryer and Iles 1972), and at 35‰ (full-strength seawater: Brock 1954; Hora and Pillay 1962; Uchida and King 1962; Canagaratnam 1966; Liao and Chang 1983). Popper and Lichatowich (1975) reported prolific reproduction of *T. mossambica* at 49‰ salinity. Some authors, however, have indicated that spawning did not occur above 30‰ (Vaas and Hofstede 1952; Fryer and Iles 1972). Optimum salinity for either enhancing or reducing reproduction by *T. mossambica* has not been determined, although Uchida and King (1962) indicated that fry production was three times higher in water of 10‰ salinity than in fresh water.

Tilapia spilurus

Osborne (1979) indicated that the growth of *T. spilurus* was not impaired in the high-salinity water of the Red Sea. Growth of *T. spilurus* and *T. aurea* × *T. spilurus* hybrids in full-strength seawater was moderate, but survival was good in Kuwait (Hopkins et al. 1985). Growth was somewhat reduced compared with that of the Taiwanese red hybrid tilapia, but the red hybrid demonstrated poor survival when water temperatures fell into the 23–24°C range during normal seasonal changes.

Additional work in Kuwait (Ridha et al. 1985) demonstrated that *T. spilurus* spawned from May to October in seawater. Fecundity in seawater was about one-half that in groundwater.

Red Hybrid Tilapia

In recent years, red tilapias have become available and have gained popularity among researchers and consumers. The popularity of red hybrids is due, in part, to their similarity in color and general body shape to such desirable marine fishes as the red snapper (*Lutjanus campechanus*). It has been widely thought that red hybrid tilapias can bring a premium price in regions where red snappers are found in the market, whereas tilapias of normal coloration would have to be priced much lower. However, consumers in some countries such as Jamaica have recognized that palatability does not differ between normal and red tilapias and have shown a distinct preference for the less-expensive form (Franklin Ross, personal communication; author, personal observations).

Galman and Avtalion (1983) reviewed the origin of red tilapia. According to those authors, Ang (in the Philippines) produced a red hybrid by crossing a female *T. mossambica* × *T. hornorum* hybrid with a male *T. nilotica*. In Taiwan, Kuo obtained reddish-orange tilapia from a cross between a mutant female *T. mossambica* of the same color and a normal male *T. nilotica*. Crosses leading to red hybrids have also been successfully made in Guam and Florida (Galman and Avtalion 1983). The latter crosses were apparently made with some combination of *T. aurea, T. mossambica,* and *T. hornorum.*

At least three of the species commonly used in the production of red hybrids, *T. mossambica, T. aurea,* and *T. nilotica,* are moderately to highly euryhaline, and the fourth, *T. hornorum,* occurs naturally in brackish water (Philippart and Ruwet 1982). Thus, it seems likely that most red hybrids would be appropriate for saline-water culture. That hypothesis is supported by at least three published studies. In one, a mutant red *T. mossambica* × normal *T. nilotica* hybrid grew more rapidly in water of 17‰ salinity than in fresh water (Liao and Chang 1983). Maturity occurred later among fish reared in brackish water, but some bruising and parasite problems were encountered that were apparently avoided in the freshwater rearing treatment. Additional work in Hawaii (Meriwether et al. 1984) and Taiwan (Watanabe 1985) has demonstrated that growth of red hybrid tilapia is more rapid in brackish water and full-strength seawater than in fresh water. An Israeli red hybrid has been developed, but it apparently lacks the salinity tolerance of those developed in Taiwan and Florida (Robert Burns, personal communication). The Israeli hybrid reportedly was not derived from *T. mossambica.*

Reports from the Bahamas (Bori Olla, Robert Wicklund, and Robert McGeachin, personal communications) indicate that red hybrid tilapias originating in the United States have performed well in full-strength seawater. Breeding of those fish is currently being conducted in a freshwater hatchery, however, because they did not spawn in full-strength seawater. That result has also been confirmed in studies of Taiwan red hybrid tilapia conducted in Kuwait (Ridha et al. 1985).

Conclusions

The species of *Tilapia* most tolerant of brackish and high-salinity waters are *T. zilli* and *T. mossambica.* The former is of little interest to aquaculturists because of its relatively slow growth and the latter has the disadvantage of reproducing at a very early age, which results in overpopulation and stunting. In addition, *T. mossambica* is less acceptable than many other tilapias in some markets because of its dark coloration and black peritoneum.

The red hybrid tilapias, which have shown growth in, and high tolerance for, saline waters, appears to be a good candidate for brackish and seawater culture systems. Their culture appears feasible in salinities up to at least 50% seawater (17.5‰).

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A Morphometrical Comparison between Cultured and Wild Juvenile American Shad[1]

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Abstract.—A morphometric comparison was made between cultured and wild American shad (*Alosa sapidissima*). Seven morphometric measurements, made with an ocular micrometer and dial caliper calibrated to 0.1 mm, were: standard length, preanal length, predorsal length, head length, eye diameter, snout length, and body depth. A linear relationship between each of the dependent variables and standard length was strongly supported by the statistical evidence. Expressed as a percentage of the means, the standard errors (SE) ranged from 1.3 to 6.9%. The different developmental environments of the two groups of fish did not produce morphometric differences in the measured characteristics; therefore, such measurements cannot be used as an aid in distinguishing cultured from wild American shad.

The sequence of egg development for American shad (*Alosa sapidissima*) has been described by Hildebrand (1963), Watson (1968) and Marcy (1976). Leim (1924) and Hildebrand (1963) described the development of larvae to the yolk absorption stage. These and other early development studies of American shad were summarized by Mansueti and Hardy (1967), Lippson and Moran (1974), Jones et al. (1978), and McGowan and Berry (1984). Johnson and Loesch (1983) described the development of American shad from the yolk absorption stage to the juvenile stage of development. The objective of this study is to statistically compare morphometric characteristics of cultured and wild (field-sampled) juvenile American shad. Differences between characteristics would be an aid in evaluating the success of American shad stocking programs in areas where natural reproduction also occurs.

Methods

Adult American shad were collected in 1979 on their spawning grounds in the Pamunkey River, Virginia, on 29 May, 6 June, and 21 June. Eggs were stripped and fertilized in the field and then transported to the Harrison Lake National Fish Hatchery, Charles City, Virginia. Eggs were cultured in a flow-through system designed by Blair (1977). Environmental control and monitoring of the culture system were described in detail by Johnson (1980). Larvae were sampled daily for the first 30 d, then weekly until the end of a 100-d period. Samples were preserved according to Berry and Richards (1973) in 10% buffered formalin. Larval development was separated into three stages associated with caudal development: preflexion, flexion, and postflexion.

Samples of wild juvenile American shad were collected with a framed 1.52 × 1.52-m pushnet (Kriete and Loesch 1980) at approximately 3-week intervals between 20 June and 9 October 1979 in the Pamunkey River. Only fish in the postflexion stage (standard length 18–30 mm) were captured. To assure that the ranges in standard length were reasonably alike for cultured and wild juveniles, only the data from postflexion fish were analyzed.

Seven morphometric measurements were taken on the left side of the body (Figure 1) with an ocular micrometer and a dial caliper calibrated to 0.1 mm. Measurements followed closely those used by Houde et al. (1974):

(1) standard length (SL): tip of the snout to the point midway between the tenth superior procurrent caudal ray and the first inferior caudal ray (Richards et al. 1974);

(2) preanal length (PAL): tip of the snout to the

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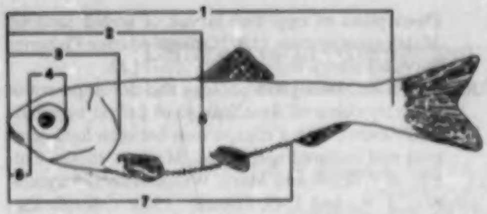

FIGURE 1.—A 28.5-mm postflexion American shad larva illustrating methods of measuring: (1) standard length, (2) predorsal length, (3) head length, (4) eye diameter, (5) body depth, (6) snout length, (7) preanal length.

end of the anus measured along the midline of the body;

(3) predorsal length (PDL): tip of the snout to the origin of the first dorsal ray measured along the midline of the body;

(4) head length (HL): tip of the snout to the posterior margin of the opercular membrane and bone;

(5) eye diameter (HED): horizontal diameter between the anterior and posterior edges of the fleshy orbit;

(6) snout length (SNTL): tip of the snout to the anterior margin of the fleshy orbit on the eye;

(7) body depth (BD): vertical height of the body measured at the origin of the first dorsal ray.

Univariate and multivariate procedures in the Statistical Analysis System (SAS 1982) were used to compare the morphometrics of cultured and wild postflexion American shad. The SAS General Linear Models procedure for analysis of covariance was used to compare the cultured and wild groups by regressing each dependent morphometric variable (PAL, PDL, HL, HED, SNTL, and BD) on the independent covariate (SL). Log transformations of the dependent and independent variables were previously found necessary to express HL, HED, SNTL, and BD as linear functions of SL (Johnson and Loesch 1983).

TABLE 1.—Means, SEs, and indices of precision (I = 100 × SE/mean) for cultured (C) and wild (W) groups of postflexion American shad.

Dependent variable	Group	Mean ± SE[a]	I (%)
Preanal length	C	16.2±0.481	2.9
(PAL)	W	15.8±0.211	1.3
Predorsal length	C	11.2±0.277	2.4
(PDL)	W	10.6±0.140	1.3
Head length	C	5.8±0.274	4.7
(HL)	W	5.4±0.112	2.0
Snout length	C	1.4±0.071	5.0
(SNTL)	W	1.3±0.049	3.7
Eye diameter	C	1.8±0.099	5.5
(HED)	W	1.8±0.054	3.0
Body depth	C	4.5±0.314	6.9
(BD)	W	4.4±0.147	3.3

[a] Means and SEs are not adjusted for the covariate.

The SAS multivariate analysis of variance was used to compare the multivariate arrays of the cultured and wild groups. The Wilks criterion (Λ) was used to examine the hypothesis of no difference between the cultured and wild postflexion groups in the multivariate arrangement.

The results of statistical analysis are reported as the probability of observing a deviation as large, or larger, solely due to chance. To avoid a declaration of significance unless the evidence was strong, we chose a conservative level of significance ($P_\alpha = 0.01$) for statistical inference.

Results and Discussion

Fifty-five postflexion American shad were analyzed, 18 cultured and 37 wild. Standard lengths ranged from 19.3 mm to 29.7 mm, and 19.4 mm to 27.2 mm for the cultured and wild groups, respectively.

The dependent variables PAL, PDL, HL, SNTL, HED, and BD were measured with a high degree of precision. Expressed as a percentage of the means, the standard errors ranged from 1.3% to 6.9% (Table 1). Thus, we felt that the power of our tests was not seriously diminished by the choice of the level of significance ($P_\alpha = 0.01$) to enhance protection against a type-I error, nor by the inequality of group replication.

A linear relationship between each of the dependent variables and SL was strongly supported by the statistical evidence (Table 2). Conversely, there was no evidence to imply significant differences between the paired regression coefficients. All differences between the respective adjusted means of the dependent variables were also nonsignificant with the exception of the BD mean difference. We suspect that the borderline significance indicated for the adjusted geometric mean difference of 0.42 mm in BD was due to stomach contents rather than to the developmental environment because the other five mean differences were nonsignificant.

Standard length values were omitted from the multivariate analysis of variance because SL was a fixed variable. In $k = 6$ space, the null hypothesis H_0 of no significant difference between the cen-

TABLE 2.—Summary of analysis of covariance test of significance between cultured and wild postflexion American shad. Dependent variables were each regressed on standard length. Rejections of the null hypothesis H$_0$: $\beta_0 = 0$ indicates the presence of regression; H$_0$: $\beta_1 = \beta_2$ states that variances are homogeneous; H$_0$: $\gamma_1 = \gamma_2$ states that adjusted group means are equal.[a]

Dependent variable	Adjusted mean differences (mm)[b]	Probability of a chance deviation	
		H$_0$: $\beta_1 = \beta_2$	H$_0$: $\gamma_1 = \gamma_2$
Preanal length (PAL)	0.130	0.191	0.630
Predorsal length (PDL)	0.296	0.095	0.077
Log$_{10}$head length (HL)	0.024	0.842	0.804
Log$_{10}$snout length (SNTL)	0.046	0.099	0.373
Log$_{10}$eye diameter (HED)	0.035	0.637	0.612
Log$_{10}$body depth (BD)	0.415	0.904	0.009

[a] For all H$_0$: $\beta_0 = 0$, $P < 0.001$.

[b] Arithmetic mean differences for PAL and PDL; geometric mean differences for the others.

troids of the cultured and wild groups was accepted ($\Lambda = 0.887$, $P = 0.304$; the Hotelling–Lawley and Pillai criteria available in the SAS routine also had $Ps = 0.304$). Discriminant analysis was not pursued since multivariate analysis of variance should first establish the existence of a significant difference (Pimental 1979).

We conclude that the different developmental environments did not produce morphometric differences in the characteristics measured; thus, such measurements cannot be used as an aid in separating cultured and wild American shad specimens.

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Differences in Morphology between Wild and Hatchery Populations of Juvenile Coho Salmon

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Abstract.—To assess the degree of morphological divergence between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*), juveniles from five hatcheries and five wild populations in south-coastal British Columbia were studied. The morphology of hatchery coho salmon was significantly less variable than the morphology of wild-reared juveniles with respect to individual characteristics and overall body form. Populations that were close geographically tended to be similar morphologically in wild–wild and hatchery–hatchery comparisons, but not when wild and hatchery populations were compared together. A principal components analysis suggested a distinct grouping of juvenile coho salmon into hatchery and wild morphological types. Discriminant function analysis and jackknifed classification procedure indicated that juvenile coho salmon could be classified as either hatchery or wild in origin with over 90% accuracy. The morphological differences and the reduced variation of hatchery juveniles observed in this study probably result, in large part, from environmental differences and the greater homogeneity of hatchery environments compared to natural rivers and streams.

Assessment is a vital part of any enhancement program for salmonids. Recently, biological assessment studies have been conducted on long term spawning channel operations for pink salmon (*Oncorhynchus gorbuscha*) and sockeye salmon (*O. nerka*) (Fraser and Fedorenko 1983; MacDonald and Hume 1984). Relatively little work, however, has been conducted on assessment of artificially produced coho (*O. kisutch*) or chinook (*O. tshawytscha*) salmon. Concern regarding hatchery production of salmonids has focused on the potential genetic alteration of populations through artificial selection of returning adults, especially when limited numbers of parents are used as broodstock (Allendorf and Utter 1979; Allendorf and Phelps 1980; Larkin 1981; Ryman and Stahl 1981). In contrast, relatively little attention is paid to the potential effects that artificial rearing conditions in hatchery ponds might have on the subsequent survival of hatchery juveniles released into the wild. Because coho and chinook salmon are the primary *Oncorhynchus* species raised in hatcheries, it should be in these species that the greatest potential for phenotypic alteration exists because the other species, when enhanced, breed in seminatural spawning channels.

Morphology is, at least in part, under genetic control (Riddell and Leggett 1981; Taylor and McPhail 1985a) and has been suggested to be of adaptive significance in fishes (Gatz 1979; Riddell and Leggett 1981; Donnelly and Dill 1984; Taylor and McPhail 1985a, 1985b). If proper assessment of hatchery practices is to be achieved, the identification of as many phenotypic differences as possible between hatchery and wild salmonids is desirable. In this manner all potential alterations to natural populations through hatchery practices can be identified and hypotheses concerning their effects on survival can be formulated and tested (Paszkowski and Olla 1985). The primary goal of this study was to document the level of divergence in morphology between wild and hatchery juvenile coho salmon.

Methods

Samples of juvenile coho salmon were collected from five south-coastal streams and from five coastal hatcheries operated by the Canadian Department of Fisheries and Oceans (Figure 1). All juveniles collected were between 40 and 60 mm standard length. Mean (±SD) standard lengths for wild (48.6 ± 7.68 mm) and hatchery (48.2 ± 5.63 mm) juveniles were not significantly different ($P > 0.05$). Hatchery juveniles were produced from wild broodstock except for the Capilano River hatchery juveniles, which were produced from hatchery broodstock. Specimens were preserved in 10% buffered formalin and stored in 37.5% isopropyl alcohol. Morphological features measured were ones for which hypotheses concerning their functional significance exist (Hubbs and Lagler 1967; Gatz 1979; Riddell and Leggett 1981; Taylor and McPhail 1985b). Initially, pectoral fin lengths were to be compared among populations but, since there

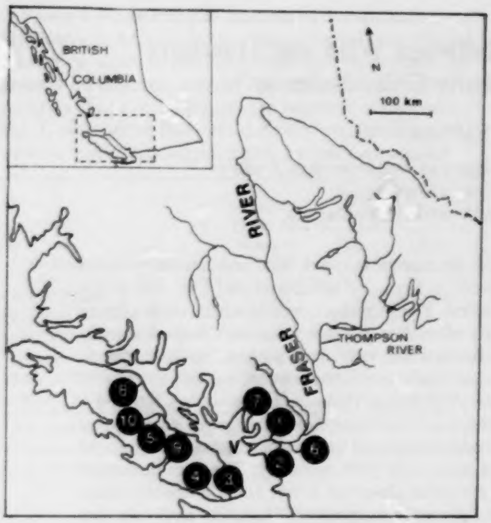

FIGURE 1.—Location of wild populations (1–5) and hatchery populations (6–10, underlined) of juvenile coho salmon sampled in the study: 1 = Silverdale Creek; 2 = Wade Creek; 3 = Demamiel Creek; 4 = Kirby Creek; 5 = Morrison Creek; 6 = Chilliwack; 7 = Capilano; 8 = Quinsam; 9 = Big Qualicum; 10 = Puntledge; V = Vancouver, British Columbia. River and creek lengths are not drawn to scale.

TABLE 1.—Adjusted[a] mean (SD) body measurements of juvenile coho salmon from wild and hatchery populations.

Character	Wild $N=197$	Hatchery $N=195$
Body depth (mm)	12.04 (0.644)	11.92 (0.533)
Caudal peduncle depth (mm)	4.95 (0.291)	4.83 (0.256)
Caudal peduncle length (mm)	7.84 (0.398)	8.16 (0.315)
Dorsal fin area (mm^2)	36.70 (6.248)	30.44 (6.120)
Anal fin area (mm^2)	32.79 (5.043)	27.08 (5.255)
Caudal fin area (mm^2)	91.15 (12.032)	76.96 (11.045)
Pelvic fin length (mm)	6.88 (0.397)	6.27 (0.328)
Snout to anterior pectoral fin (mm)	12.60 (0.518)	12.10 (0.384)
Snout to anterior pelvic fin (mm)	26.29 (0.782)	26.19 (0.611)
½(body depth + maximum body width) (mm)	9.41 (0.481)	9.47 (0.516)
Fineness ratio	5.22 (0.269)	5.18 (0.278)
Head length (mm)	13.98 (0.489)	13.65 (0.415)
Head depth (mm)	9.14 (0.421)	8.70 (0.312)

[a] Adjusted to 48 mm standard length.

was widespread occurrence of damaged pectoral fins in the hatchery samples, I discarded this character. All length and depth measurements were made to the nearest 0.01 mm with Helios calipers and followed procedures of Hubbs and Lagler (1967). Median fin areas were determined from outline sketches made of the fins with a camera lucida. The areas of resultant sketches were determined by the use of an Apple II microcomputer digitizing tablet.

To minimize differences in measurements due to differences in fish length, all measurements were adjusted to the overall sample mean standard length (pooled wild and hatchery fish) (Gould 1966; Riddell and Leggett 1981). Logarithmically transformed measurements (base 10) were regressed against the logarithm of standard length in each sample and the resultant slopes were used to calculate an adjusted data set (Steel and Torrie 1980). All morphological data used in the subsequent analyses were the antilogs of the adjusted data.

Analyses of adjusted data consisted of multivariate analysis of variance (MANOVA) and principal components analysis of the pooled data set with MIDAS (Fox and Gulre 1976). Multivariate analysis of variance was used to test for significant differences in body form among populations, and the principal component analysis was used to see if a hatchery–wild grouping existed with respect to morphology. Box's test (Fox and Gulre 1976) was used to test for equality of variance–covariance matrices. In addition, simple correlation coefficients (Steel and Torrie 1980) were calculated between geographic distances separating the mouths of rivers with wild populations (or mouths of rivers on which hatcheries were located) and morphological distances among populations. A cartometer was used to determine the geographic distances between river mouths from topographic maps. Morphological distance was expressed as the Mahalanobis distance (D^2) among multivariate means (Fox and Gulre 1976).

Results

At a standard length of 48 mm, juvenile coho salmon from hatchery populations were morphologically less variable than juveniles from wild populations (Table 1). The proportion of population coefficients of variation that ranked below the overall wild–hatchery coefficient of variation for each characteristic was significantly greater among hatchery populations (pooled $\chi^2 = 24.1$, $P < 0.001$; heterogeneity $P > 0.1$) yet not among wild populations (pooled $\chi^2 = 0.31$, $P > 0.1$; heterogeneity $P > 0.1$). Sample centroids were significantly different (MANOVA, maximum $P < 0.001$) in all pairwise comparisons (Table 2). Wild–

TABLE 2.—Matrix of Mahalanobis D^2 among wild and hatchery samples of juvenile coho salmon in the study.[a] All comparisons are significant ($P < 0.001$).

	Wild					Hatchery			
Group[a]	1	2	3	4	5	6	7	8	9
Wild									
2	7.4								
3	6.2	16.7							
4	10.0	16.8	5.2						
5	12.2	3.5	19.4	19.8					
Hatchery									
6	12.3	6.1	20.3	23.5	5.4				
7	16.4	7.6	31.5	33.2	12.1	6.9			
8	18.9	21.1	16.1	21.8	25.6	16.1	23.4		
9	15.1	17.9	17.3	19.4	24.2	16.4	19.5	9.1	
10	25.0	26.9	23.7	31.7	34.9	23.4	21.5	7.4	8.4

[a] Creeks: 1 = Silverdale; 2 = Wade; 3 = Demamiel; 4 = Kirby; 5 = Morrison.
Hatcheries: 6 = Chilliwack; 7 = Capilano; 8 = Quinsam; 9 = Big Qualicum; 10 = Puntledge.

wild comparisons (mean $D^2 = 11.7$) tended to be less distinct than did hatchery–hatchery comparisons (mean $D^2 = 15.2$). The greatest distinction appeared to be in wild–hatchery comparisons of centroids (mean $D^2 = 20.4$).

The correlation matrix was used to calculate the variable coefficients for the first three principal components (PC), which together accounted for 81% of the total morphological variation (Table 3). A plot of the scores of PC 1 and PC 2 suggests considerable morphological separation of wild and hatchery juveniles (Figure 2) and slight but detectable separation when the scores for PC 2 and PC 3 are plotted (Figure 3). The mean scores of hatchery and wild juveniles were significantly different for PC 1, PC 2, and PC 3 (ANOVA, $P < 0.0001$) yet not for PC 4 ($P > 0.05$). A multivariate analysis of variance of scores from the first three PC axes was highly significant ($P < 0.0001$) for differences in the hatchery and wild centroids.

The above procedures suggest that a grouping of hatchery and wild juveniles into distinct morphotypes exists and a two-group MANOVA with the original adjusted variables indicated a significant difference between wild and hatchery means ($P < 0.0001$). I conducted a two-group discriminant function analysis (Dixon 1981) to identify which morphological features "best" differentiated wild from hatchery juveniles. The generalized variance (a composite of all characters) was significantly lower in the hatchery pooled sample ($P <$

TABLE 3.—Variable coefficients for the first three principal components (PC) describing morphological variation in juvenile coho salmon ($N = 392$).

Variable	PC 1	PC 2	PC 3
Body depth	0.255	−0.419	0.198
Caudal peduncle depth	0.260	−0.113	0.340
Caudal peduncle length	−0.072	−0.259	0.121
Dorsal fin area	0.335	0.160	−0.296
Anal fin area	0.332	0.212	−0.001
Caudal fin area	0.349	0.179	−0.255
Pelvic fin length	0.254	0.194	0.296
Snout to anterior pectoral fin	0.239	0.127	0.445
Snout to anterior pelvic fin	0.128	−0.041	0.062
½(body depth + maximum body width)	0.237	−0.517	−0.169
Fineness ratio	−0.239	0.515	0.174
Head length	0.202	0.035	0.348
Head depth	0.298	0.039	0.211
Cumulative % of total variance	54	69	81

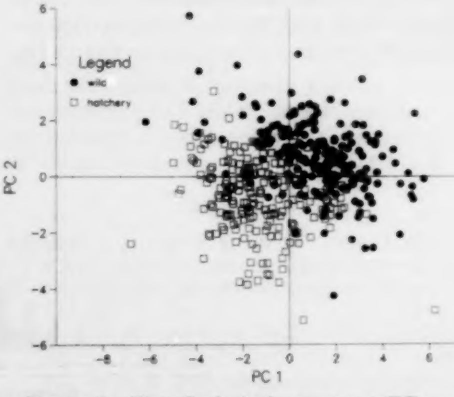

FIGURE 2.—Plot of principal component (PC) scores for PC 1 and PC 2 derived from morphological analyses of juvenile coho salmon from hatchery and wild populations.

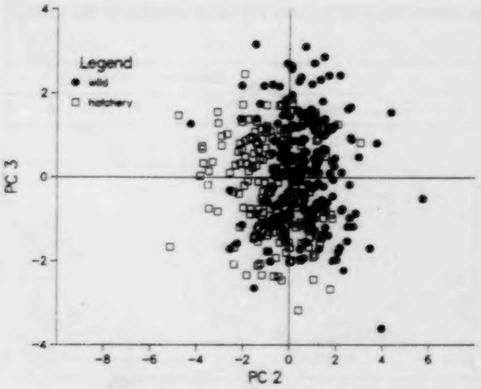

FIGURE 3.—Plot of principal component (PC) scores for PC 2 and PC 3 derived from morphological analyses of juvenile coho salmon from hatchery and wild populations.

0.001) compared to the wild pooled sample (Figure 4). I used a jackknifing classification procedure (Lachenbruch and Mickey 1968) to test the performance of the discriminant analysis. Jackknifing attempts to classify each individual based on a discriminant model calculated in the absence of the individual being classified and thus removes the bias of classifying individuals used to construct the model. Overall, 92% of the juveniles could be correctly identified as either wild or hatchery in origin by this procedure. Hatchery juveniles, however, were identified with slightly greater accuracy than were wild juveniles (93 versus 90%). Eight of the 13 morphological characteristics were entered into the discriminant model; caudal fin area, pelvic fin length, head depth, fineness ratio, and caudal peduncle length were the characters that best discriminated between wild and hatchery juveniles.

TABLE 4.—Classification of "unknown" hatchery and wild coho salmon juveniles from various wild populations and Oregon State hatchery populations. Classification decision discriminant score is −0.005. Asterisk (*) indicates classification error.

Population and location	Discriminant score	Classification
Hatchery		
Big Creek	−0.22	Hatchery
Big Creek	−1.12	Hatchery
Cole River	0.02	Wild*
Salmon River	−1.30	Hatchery
Salmon River	−0.21	Hatchery
Wild		
Texada Island		
Gillies Bay Creek	1.62	Wild
Queen Charlotte Islands		
Graham Island	0.36	Wild
Graham Island	0.31	Wild
Vancouver Island		
Hammond Bay Creek	1.99	Wild
Upper Fraser River		
Louis Creek	1.92	Wild
Columbia River		
Salmon River	3.11	Wild

As a further test of accuracy of the discriminant analysis, specimens from Oregon State hatcheries and from other wild populations were analyzed and processed by means of the hatchery–wild discriminant function. These "unknowns" were classified as either hatchery or wild in origin with over 90% accuracy (Table 4).

Morphological distance (D^2) was positively correlated with geographic distance between river mouths for the wild samples ($r = 0.71$, 8 df, $P < 0.05$) and for the hatchery samples ($r = 0.60$, 8 df, $0.1 > P > 0.05$) although the latter correlation was not significant. The correlation between morphological and geographic distances among hatchery

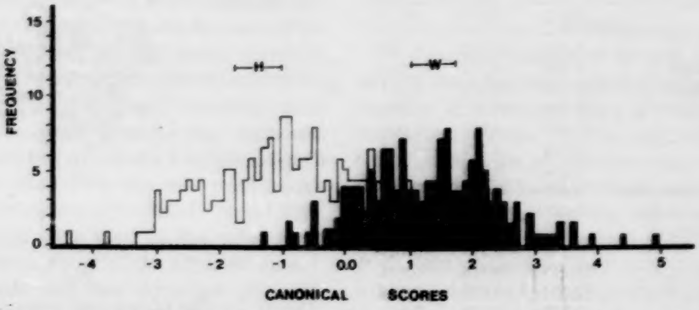

FIGURE 4.—Two-group discriminant function analysis describing morphological separation between hatchery (open bars, $N = 195$) and wild (solid bars, $N = 197$) juvenile coho salmon; W = wild group mean (±95% confidence interval), H = hatchery group mean (±95% confidence interval).

and wild samples, however, was low and not significant ($r = 0.17$, 23 df, $P > 0.4$). Thus, although geographic proximity was reflected in morphological similarity among the wild samples and among the hatchery samples, no such trend was evident when hatchery and wild populations were compared together.

Discussion

The results of this study suggest that hatchery rearing practices result in morphological changes in juvenile coho salmon relative to wild juveniles. Is the morphological divergence between wild and hatchery juvenile coho salmon a genetic effect and perhaps reflective of adaptive differences? The available evidence indicating a genetic component to morphological variation in salmonids (Ricker 1972; Riddell and Leggett 1981; Taylor and McPhail 1985a) may suggest some component of this divergence is genetic in origin but, in the absence of breeding data in this study, no assignment of the observed phenotypic differences to genetic differences is justified. Further, since all but one of the hatchery populations studied originated from wild brood stock, the morphological divergence between wild and hatchery juveniles probably reflects, in large part, environmental differences. In addition, the reduced phenotypic variance within the hatchery samples is probably largely due to the greater homogeneity of hatchery environments relative to environments from which the wild juveniles were obtained (Hynes et al. 1981). Whether or not the morphological differences observed have any significance to performance in the wild is of interest in light of available evidence (Gatz 1979; Riddell and Leggett 1981; Taylor and McPhail 1985b), but firm conclusions would require functional experiments on wild and hatchery juveniles.

Individuals from populations of salmonids that are geographically close tend to be similar morphologically (McIntyre 1976; Hjort and Shreck 1982; Taylor and McPhail 1985a), yet there was no correlation between the morphological characteristics of hatchery and wild juveniles from populations that were close geographically. The extended rearing of juveniles in the hatchery environment may reduce the tendency for proximal populations to be similar morphologically when one population is maintained in a hatchery. The lack of a correlation between geographic and morphological distances among proximal hatchery and wild populations supports the separation of wild and hatchery-reared juveniles into distinct types and may indicate that the hatchery juveniles lack the "locally adapted" morphological characteristics of juveniles from wild populations, at least while they are in the hatchery environment.

Morphological divergence between wild and hatchery juvenile coho, however, does not necessarily imply the changes are deleterious. The hatchery-reared juveniles may simply be responding to different environmental conditions in the hatchery. Further, because the hatchery coho salmon in this study are largely reared to smolts, the morphological differences that I have described may have little relevance upon release into the wild. A similar degree of divergence, however, may exist between hatchery and wild yearling coho salmon and morphological differences are but part of a range of phenotypic change that may accompany hatchery rearing (Vincent 1960; Dickson and MacCrimmon 1982). The real question posed by this analysis is—if morphological changes occur when juveniles are reared in a hatchery environment, how temporary are these changes and how do they affect the survival of hatchery juveniles released into the wild? Do hatchery juveniles become indistinguishable from wild juveniles upon release into a river or stream after a few hours? a week? or several months? The concept of a time scale for what may be temporary, environmentally induced phenotypic differences should be an important area of future research because it has been amply demonstrated that hatchery and wild juvenile salmonids are phenotypically different and that mortality of hatchery-reared salmonids may be rapid following release (Shetter and Hazzard 1941).

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Impact of Paddlefish on Plankton and Water Quality of Catfish Ponds

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Abstract.—The effects of paddlefish (*Polyodon spathula*) on zooplankton, chlorophyll *a*, total ammonia nitrogen, and nitrite in a yearling paddlefish–catfish polyculture were measured in eight 0.04-hectare ponds. All ponds were stocked with channel catfish (*Ictalurus punctatus*) and the hybrid, channel catfish × blue catfish (*Ictalurus furcatus*), at commercial stocking rates and four ponds were stocked with paddlefish at a rate of 990/hectare. In the paddlefish treatment, zooplankton densities were significantly lower, particularly during the period March through May. Some recovery of the zooplankton occurred June to September. Seasonal mean chlorophyll-*a* concentrations were significantly higher in the paddlefish treatment, apparently because of reduced zooplankton grazing pressure. Dissolved nitrogen was related inversely to chlorophyll-*a* concentrations. The unchecked growth of the algal community (particularly of colonial forms) in the paddlefish treatment apparently interfered with efficient feeding of paddlefish on available zooplankters.

The paddlefish (*Polyodon spathula*) has drawn attention as a possible culture species (Swingle 1965; Semmens 1982). Early investigators established that paddlefish were primarily planktivores and insectivores (Kofoid 1900; Eddy and Simmer 1929). Rosen and Hales (1981), in their study of adult paddlefish in the unchannelized Missouri River, found the fish to be an indiscriminant filter feeder on zooplankton. The fish appeared to feed day and night and digestion rates were slow.

From data presented by Purkett (1963) and Swingle (1965), we calculated mean paddlefish growth rates at about 10.5 g (1.6 mm) and 27.0 g (1.2 mm)/d, respectively. In both studies, a few adult paddlefish, some as large as 4.0 kg, were stocked into catfish ponds that received inorganic fertilizer and feed. Michaletz et al. (1982) studied the feeding ecology and growth of young-of-the-year paddlefish in hatchery ponds. Paddlefish stocked at rates of 50,000–60,000 fry per hectare grew rapidly, averaging 2.4 mm/d, while selectively feeding on large *Daphnia*. With the decline of the *Daphnia* population, growth rate declined but increased again to 1.0 mm/d when fish converted to filter feeding at a total body length of 120 mm. Filter feeding fish fed on all zooplankton in the pond; however, they appeared to select the slower moving rotifers, cladocerans, and cyclopoid copepods.

Objectives of our study were to determine the impact of paddlefish on plankton communities and on selected water quality variables in a yearling paddlefish–catfish polyculture.

Methods

Eight 0.04-hectare ponds were stocked with equal numbers of channel catfish (*Ictalurus punctatus*) and a catfish hybrid, channel catfish × blue catfish (*Ictalurus furcatus*), at a combined rate of 7,410 fish per hectare. Four of the ponds were randomly chosen and stocked with paddlefish at a rate of 990/hectare. Catfish were stocked at a mean weight of 12 g on 7 March 1982 and paddlefish at a mean weight of 140 g (38 cm) on 10 March 1982. Fish were fed a 32% protein, floating fish feed daily. Feeding rates were adjusted for fish growth and each pond received the equivalent of 5,126 kg feed/hectare during the 203-d culture period.

Fish sampling.—Each month, May to August, four paddlefish were seined from treatment ponds for stomach content analysis. Each fish was pithed and the gut removed and placed in 10% formalin. The collected fish were replaced with fish of comparable size. Stomach contents of each fish were measured volumetrically and examined with a dissecting microscope to determine gross characteristics and to identify large items (Laevastu 1965). A 1-mL aliquot of the stomach contents was diluted to 100 mL with water and a portion was examined in a Sedgewick-Rafter cell with a compound microscope. Twenty-five randomly selected fields were examined to estimate the volume that each food category contributed to the stomach contents (Laevastu 1965). The mean volume percent that each category contributed to the total volume of food for that sample date was then tabulated (Wallace 1981). Intestinal material was examined to determine extent of digestion.

Plankton and water quality.—Water was collected weekly for chlorophyll *a*, nitrogen, and plankton analyses. A composite water sample from each pond was obtained with a column sampler (Boyd 1979). Water column samples were taken from 10 representative areas of each pond and pooled. For zooplankton samples, 10 L of this composite water sample were passed through an 80-μm-mesh, Wisconsin-style plankton net and the concentrate preserved in 5% formalin. Zooplankton were counted in a Sedgewick-Rafter cell with a compound microscope (Lind 1979). Cladocerans and rotifers were identified to species and copepods to suborder by use of current taxonomic references. All samples were examined qualitatively. Zooplankton counts were made at monthly intervals on samples from all ponds. Weekly counts were made during periods when examination revealed notable qualitative or quantitative changes in the zooplankton community.

Chlorophyll *a* was extracted from water samples with acetone and measured spectrophotometrically (APHA et al. 1980). Dissolved oxygen and temperature were measured in situ with a Yellow Springs Instrument Company polarographic dissolved oxygen meter and thermistor. Total ammonia nitrogen was measured by the phenate method (Boyd 1979). Nitrite concentration was determined by the colorimetric method (APHA et al. 1980).

Mean values of chemical and biological variables were statistically compared by means of analysis of variance and Student–Newman–Keuls tests, and correlations were derived from the Statistical Analysis System package (Helwig and Council 1979). Statements of significant differences were made based on $P = 0.05$.

Results

Cladoceran standing stocks were significantly lower in paddlefish ponds from March through May (Figure 1). From June through September cladoceran densities in the two treatments were similar though subject to wide fluctuations. Succession among dominant cladocerans in March through May differed markedly between treatments (Figure 2). In paddlefish ponds *Bosmina longirostris* and *Daphnia* sp. disappeared earlier and *Diaphanosoma brachyurum* was not found. However, during the summer, succession in the two treatments was similar. *Bosmina longirostris* reappeared and *D. brachyurum* appeared in the paddlefish treatment ponds and both treatments supported populations of *Moina micura* and *Ceriodaphnia rigaudi.*

Standing stocks of copepods in paddlefish ponds were significantly lower throughout the study than stocks found in control ponds (Figure 1). Beginning in mid-July, numbers of copepods rose in the paddlefish treatment but never approached densities in the control ponds.

Rotifer densities varied widely in both treatments (Figure 1). There were no significant differences in treatment means for the growing season, but in early April rotifer densities were significantly higher in the paddlefish ponds. This trend persisted throughout the remainder of April and May although the differences were not significant. Depressed rotifer densities in control ponds during this period probably resulted from competition with the abundant cladocerans (Lynch and Shapiro 1981). Succession of dominant rotifers in ponds of the two treatments (Figure 2) indicates that the paddlefish did affect species composition of the rotifer community.

Mean chlorophyll-*a* concentrations in the paddlefish treatment ponds were significantly higher than the concentrations in the control ponds (Figure 3). Trends in ammonia and nitrite concentrations in the two treatments were similar (Figure 3). Observed means of both were higher in control ponds from April through mid-July when concentrations in the paddlefish ponds rose to similar levels. Nitrite concentrations were significantly higher in the control treatment on seven of nine sampling dates in April and May.

In May, detritus and benthic organisms comprised an estimated 80% of the stomach contents of sampled fish, indicating benthic feeding (Table 1). Benthic organisms identified included ostracods, chironomids, and water mites. In addition, the meroplanktonic midge *Chaoborus* and the benthic cladoceran *Alona quadrangularis* were found. In June, planktonic algae and cladocerans made up 45 and 30% of the stomach contents by volume, respectively. In the July and August samples, fish stomachs contained 90 and 60% phytoplankton, respectively. Phytoplankton ingested were mainly *Coelastrum*, a green colony, and *Microcystis*, a blue-green colony. Examination of the posterior gut contents revealed no evidence of digestion of these algae.

Discussion

Paddlefish can be expected to alter plankton and water quality through their predation on zoo-

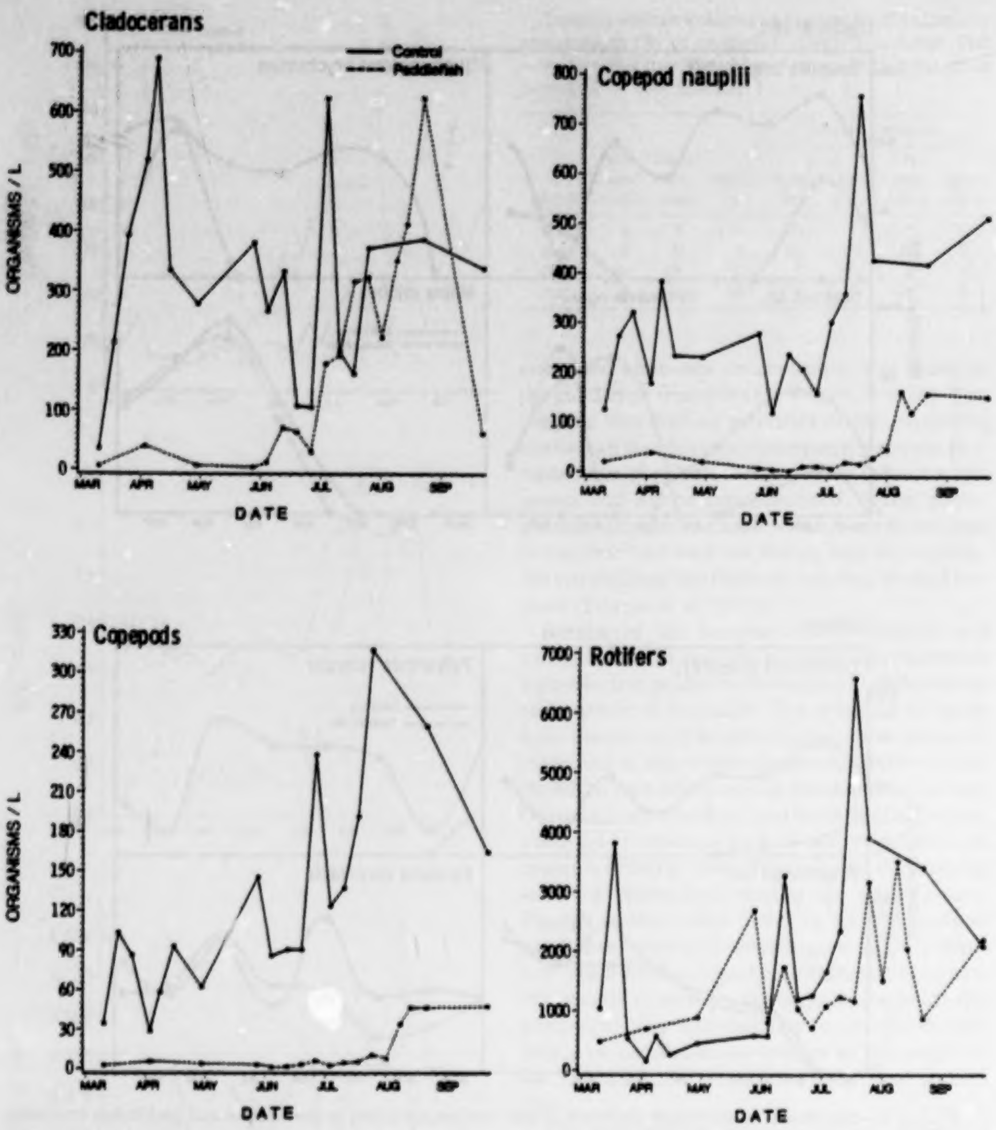

FIGURE 1.—Mean densities of cladocerans, copepods, copepod nauplii, and rotifers in the control and paddlefish treatment ponds of the polyculture study.

plankton. By dividing this study into two general periods, the effects of paddlefish predation can be seen. During the early part of the study, zooplankton in paddlefish ponds were depressed when compared to the control ponds. The later part of the study was characterized by a recovery of crustacean zooplankton, particularly the cladocerans, which reached densities found in the control ponds.

Trends in chlorophyll *a*, ammonia, and nitrite were different for these two periods. During the low zooplankton period, chlorophyll *a* rose rapidly and ammonia and nitrite levels were low when

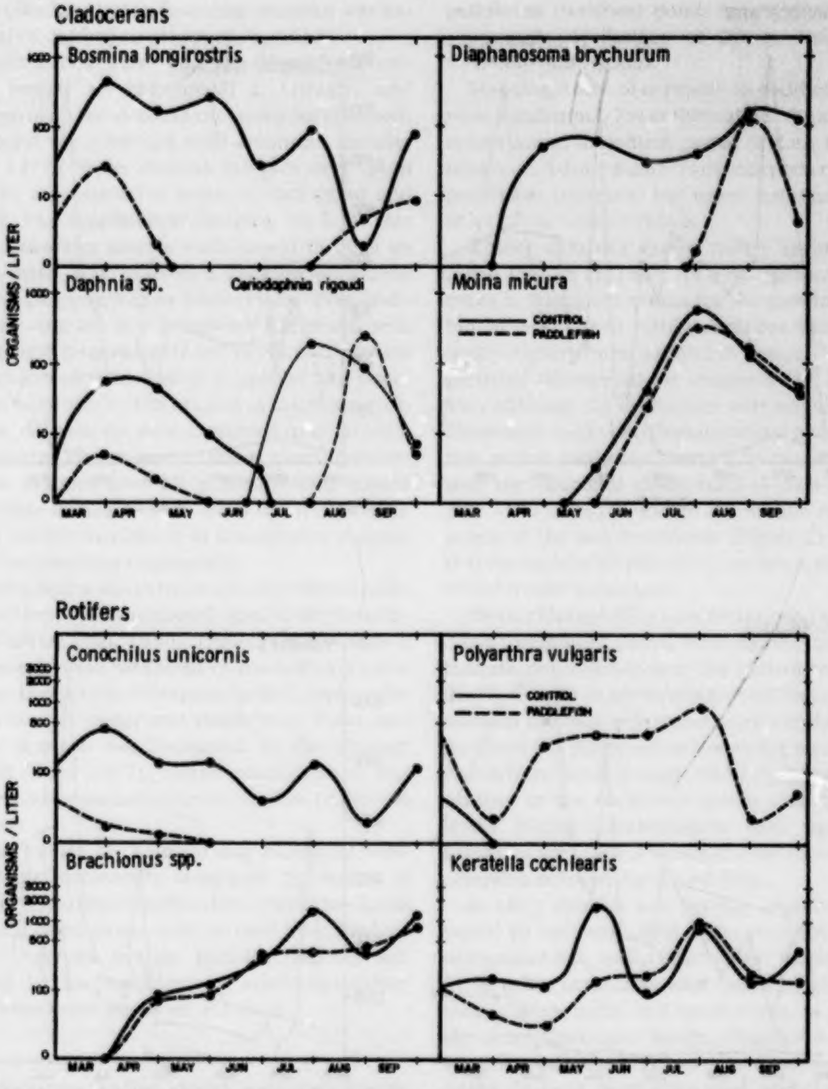

FIGURE 2.—Succession of dominant cladocerans and dominant rotifers in the control and paddlefish treatment ponds of the polyculture study.

compared to the control. During the period of crustacean zooplankton recovery, chlorophyll *a* leveled off and ammonia and nitrite concentrations increased. Research has shown that removal of crustacean zooplankters can result in an order of magnitude increase in phytoplankton standing stock (Andersson et al. 1978; Lynch and Shapiro 1981). Opuszynski (1981) and Burke et al. (in press) reported significantly ($P < 0.05$) higher chlorophyll-*a* concentrations in ponds stocked with planktivorous carps that suppressed zooplankton throughout the growing season. This effect is apparent in the paddlefish treatment ponds during the early part of our study.

The recovery of the cladoceran population coincides with the leveling off of chlorophyll *a* and the rise of ammonia and nitrite concentrations. A significant correlation between number of cladoc-

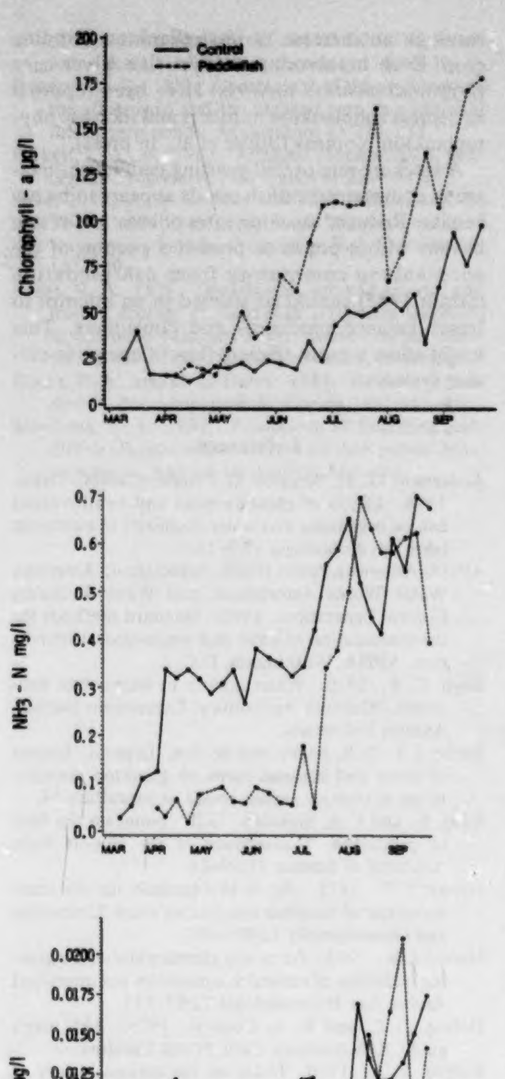

FIGURE 3.—Mean weekly concentrations of chlorophyll *a* (µg/L), ammonia nitrogen (mg/L), and nitrite nitrogen (mg/L) in the control and paddlefish treatment ponds of the polyculture study.

TABLE 1.—Mean volumes and estimates of volumetric composition (%) of paddlefish stomach contents. Fish were sampled monthly, May–August, from polyculture ponds. (T = trace amounts.)

				Animal material			
Month	Volume (mL)	Plankton algae	Detritus	Rotifers	Copepods	Cladocerans	Benthos
May	4	T	50	10	5	5	30
Jun	8	45	5	10	10	30	T
Jul	10	90	T	5	T	5	T
Aug	8	60	10	5	5	20	T

erans and ammonia concentration was found in the paddlefish treatment ($R = 0.79$, $N = 16$). This suggests that feeding activities of the expanding crustacean zooplankton community decrease phytoplankton populations and in turn influence ammonia and nitrite concentrations. When phytoplankton populations are low, nutrients accumulate in the dissolved state but during high phytoplankton populations, nutrients accumulate as algal biomass (Tilman et al. 1982).

Results of the zooplankton inventories and stomach content analyses revealed that paddlefish were effective predators on zooplankton during the early period of the study. The presence of significant quantities of benthic organisms in stomachs examined in May demonstrates the ability of paddlefish to feed effectively at the sediment surface. Crustacean zooplankton, particularly cladocerans, made up as much as 80% of individual stomach contents of fish in June. This reduced the standing stock of crustaceans during the early period. Though rotifers were found in the stomachs of paddlefish, apparently affecting community structure, there were no significant differences in quantity of rotifers between the control and paddlefish treatment. This may reflect a lower capture efficiency by paddlefish for rotifers or the ability of rotifer populations to increase their numbers rapidly.

The zooplankton recovery period suggests a change in paddlefish feeding or secondary production in excess of fish demand. The latter explanation seems unlikely because paddlefish growth (2 g/d) in this study appears slow compared to the rapid growth capacity of paddlefish as demonstrated by growth of sparsely stocked paddlefish in catfish culture ponds (Purkett 1963; Swingle 1965). In addition, paddlefish stomachs contained high percentages of apparently indigestible planktonic algae rather than preferred food items.

A possible explanation of the recovery of zooplankton in this study is given by Rosen and Hales (1981). They reported that, in the unchannelized Missouri River, paddlefish feeding activity peaked in spring and fall and ceased altogether from late June to early September. Three explanations were given for the lack of summer feeding: high water temperature, excessive waterborne sand load, and low food density. Mean weekly water temperatures during the summer of our study varied between 25 and 31°C. Although the majority of fish examined in this study had full stomachs and intestines in summer, one fish from the August sample had an empty digestive tract which was tightly contracted as described by Rosen and Hales (1981), and one fish sampled in July had an empty intestine and full stomach. As paddlefish are reported to have slow digestive rates and to feed continuously (Rosen 1976), this suggests cessation and resumption of feeding.

An alternative explanation for the cessation of effective paddlefish predation on available zooplankton has to do with development of dense phytoplankton blooms during summer months. The digestive tracts of most fish were tightly packed with relatively large colonial algae, which were apparently passing through the gut undigested. Fish that contained at least 50% phytoplankton appeared to have bloated stomachs and intestines and mean stomach volumes of 11 mL. Those fish consuming at least 50% zooplankton had mean stomach volumes of 5 mL. *Coelastrum* and *Microcystis*, the algal genera found filling the guts of the paddlefish in our study, have both been reported to pass through the gut of fishes undigested (Yashouv and Chervinski 1961; Rabe et al. 1973). *Tilapia nilotica*, a highly evolved planktonic herbivore with an unusually low stomach acid pH of 1.4, can lyse blue-green algae cells and digest their contents (Moriarty 1973). Paddlefish apparently cannot, and ingestion of large quantities of algae may have interfered with effective feeding on zooplankton.

Management Implications

When the use of filter-feeding fish in culture ponds is considered, the impact of the fish on zooplankton communities must be anticipated. Zooplankters are voracious consumers of phytoplankton. Haney (1971, 1973) reported that zooplankton grazers filtered the entire volume of water in a eutrophic lake as many as 4.7 times during one day. Cladocerans usually accounted for 80% of the community grazing rate. The suppression of zooplankton communities by filter-feeding fishes can result in an increase in phytoplankton standing crop. Even herbivorous filterers like silver carp (*Hypothalmichthys molitrix*) have been reported to depress zooplankton numbers and increase phytoplankton biomass (Burke et al., in press).

A stocking rate of 990 yearling paddlefish/hectare in commercial catfish ponds appears to be excessive. Reduced stocking rates or creation of safe havens within ponds to protect a portion of the zooplankton community from fish predation (Smith 1985) should be studied in an attempt to better balance producers and consumers. This might allow a more efficient flow of energy in culture systems.

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COMMUNICATIONS

Growth Comparisons of Diploid and Triploid Grass Carp under Varying Conditions

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Abstract.—Growth of fry and fingerling diploid and triploid grass carp (*Ctenopharyngodon idella*) was evaluated under a variety of experimental situations that involved high and low stocking densities and various levels of food availability. Diploid grass carp grew faster and had significantly ($P < 0.05$) higher condition factors in every situation where they were grown together with triploid grass carp. When diploid and triploid grass carp were grown in separate pools and fed to satiation with duckweed, there were no significant differences ($P < 0.05$) in growth, condition factors, food conversions, or rates of food consumption. The difference in the rate of growth between diploid and triploid grass carp can be exploited to presegregate diploid from triploid fish, thus reducing the time spent certifying ploidy with a Coulter Counter®.

Importation and use of grass carp (*Ctenopharyngodon idella*) as a biological control agent for aquatic weeds has been limited in many areas, especially in North America, because of the species' potential for unwanted reproduction (Miller and Decell 1984). The recent development of triploid grass carp (generally considered to be sterile) provides a viable alternative to diploid grass carp in areas where reproduction is unwanted.

Induction of triploidy in grass carp or grass carp hybrids (female grass carp × male bighead carp *Hypophthalmichthys nobilis*) has rarely exceeded 90% on a consistent basis and can range from less than 1% to an estimated 100% with the same or similar inducements (Cassani et al. 1984; Cassani and Caton 1985). Use of triploid hybrid grass carp has generally been curtailed due to problems associated with growth, food consumption, and survival (Osborne 1982; Cassani and Caton 1983; Shireman et al. 1983). In Florida, the ploidy of all triploid grass carp must be certified with the use of a Coulter Counter® or similar instrument before any fish are stocked for aquatic weed control. Presegregation of triploid from diploid individuals based on morphological comparisons is not practical; the animals look too similar. Ploidy testing of fish populations with low percentages of triploids can be very time-consuming.

The purpose of this study was to determine what environmental factors could create a differential growth rate between diploid and triploid grass carp. If triploid fish could be reliably and practically segregated from diploid fish based on a difference in growth or condition, this would increase the efficiency of final ploidy determinations with a Coulter Counter.

Methods

Total lengths and weights of diploid and triploid grass carp were evaluated under a variety of conditions to determine which environmental factors affect growth and to what degree.

Low density: fry.—A population of grass carp from a single spawn (14–25 mm total length), estimated to be approximately 50% triploid, was introduced into a fertile nursery pond at the rate of approximately 44,000 fry/hectare. Fish were fed finely ground formulated feed (32% protein) initially. They were provided with generous quantities of duckweed (*Wolffia* sp. and *Lemna* sp.) when they reached 40–50 mm in total length (about 13 d into the 53-d test period). At the end of the test period, the fish were weighed and measured. Ploidy was determined by extracting a small volume of erythrocytes from the caudal artery and testing the cells with a Coulter Counter (Wattendorf 1986).

High density: fingerlings.—Approximately 6,000 grass carp fingerlings, 60–100 mm in total length, were stocked in a nursery pond at a rate of 267,000/hectare. These fish were fed duckweed several times a week at a rate considerably less than that required for sustained growth. The food was completely consumed in 6–8 h. During a 7-week period, weights and total lengths were determined for 36 triploid and 80 diploid individuals that had been randomly selected. Ploidy was determined with a Coulter Counter.

Low density: fingerlings.—Fifty diploid and 50 triploid grass carp fingerlings, 130–160 mm in total length, were stocked together in a pond at a rate of 4,400/hectare. Fish were fed duckweed 5 d/week. The quantity fed was somewhat less than satiation (100% consumption in 12–15 h). Total lengths and weights were determined for individual fish at the beginning and at the end of the 75-d test period. Ploidy was determined with a Coulter Counter.

Pool study: fingerlings.—Fifty diploid and 50 triploid grass carp fingerlings, averaging 130–175 mm in total length, were grown separately in 3.6-m-diameter (7,500-L) greenhouse pools. Both pools were maintained in the same manner, involving aeration, a flushing rate of one water exchange every 15 h, and periodic treatments with a malachite green–formaldehyde solution as a prophylactic against external parasites and pathogens. Fish in the pools were fed to satiation 6 d/week with duckweed (*Lemna* sp.) and were acclimated to pool conditions for 6 d before the study began. This segment of the study was conducted concurrently with the low-density fingerling segment. Fresh duckweed was weighed after it was spun in a washing machine at approximately 500 revolutions/min for 5 min. Duckweed dry weight was determined after 10 samples of preweighed fresh duckweed were dried in an oven at 70°C for 48 h.

Total lengths and weights of individual fish were recorded initially and after the next six 2-week periods. Fish were anesthetized with tricaine methane sulfonate (MS-222) prior to length and weight measurements. A YSI model 57 dissolved-oxygen meter was used to record oxygen and temperature in pools and ponds during the early morning and afternoon daily. Means for the several variables were compared between diploid and triploid fish groups by two-sample t-tests ($P < 0.05$).

Results

Low Density: Fry

Mean total lengths of diploid and triploid grass carp fry (14–25 mm), grown together in the same pond for 53 d at a relatively low density, were significantly different ($P < 0.05$). Length-frequency distributions were determined for random samples of 58 diploid and 58 triploid fish (Figure 1). Mean total lengths (±SD) were 104.6 ± 18.7 mm for diploid fish and 83.3 ± 15.3 mm for triploid fish. Mean weights (±SD) were 16.9 ± 8.4 g and 7.8 ± 4.7 g for diploid and triploid fish, respectively. Diploid fish also had a significantly higher mean condition factor (1.32 ± 0.16) than triploid fish (1.19 ± 0.17). Regressions of length (L, mm) against weight (W, g) produced from these data confirmed that diploid fish were in better condition:

triploid,

$$\log_{10} W = -5.311 + 3.205 \log_{10} L; \quad r = 0.970;$$

diploid,

$$\log_{10} W = -5.827 + 3.468 \log_{10} L; \quad r = 0.987.$$

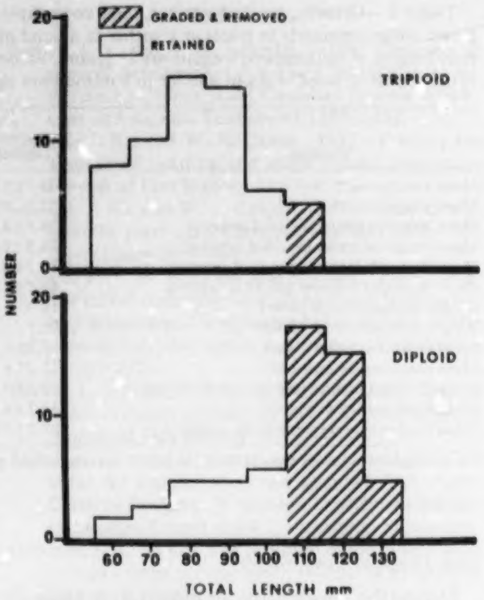

FIGURE 1.—Length-frequency distributions for 58 diploid and 58 triploid grass carp grown together for a 53-d period. Grading and removing fish longer than 105 mm (hatched area) decreased the number of diploid fish by 63.8%.

Mean pond temperature and dissolved oxygen concentration during the experimental period were 29.3 ± 1.7°C (SD) and 5.9 ± 3.4 mg/L, respectively.

High Density: Fingerlings

In the high-density, sparse-food situation, the mean total length of diploid fish (92.3 ± 16.9 mm) was again greater than that of triploid fish (80.5 ± 6.4 mm) but not significantly so ($P > 0.05$). However, the mean diploid condition factor (1.08 ± 0.08) was significantly greater than the triploid condition factor (0.95 ± 0.08). During this period, mean temperature and dissolved oxygen concentration were 28.1 ± 2.4°C and 8.1 ± 3.0 mg/L, respectively.

TABLE 1.—Growth, condition factor, food consumption, and food conversion of diploid and triploid grass carp grown either separately in pools or together in a pond at low density for 75 d. Values are means ± SD; lengths are total lengths in millimeters; weights are in grams. Values along a row (and in columns for mean condition factors) within pools or pond without a letter in common are significantly different ($P < 0.05$).

Variable	Segregated in pools		Unsegregated in pond	
	Diploids	Triploids	Diploids	Triploids
Mean length—start	138.7±13.5 z	152.1±7.1 y	133.6±8.1 z	149.1±7.0 y
Mean weight—start	33.7±10.5 z	44.6±13.5 y	28.6±5.7 z	44.1±6.3 y
Mean length increase per 14-d interval	23.7±4.9 z	20.7±7.3 z		
Mean weight increase per 14-d interval	36.3±18.9 z	34.2±14.2 z		
Mean % length increase per 14-d interval	13.2±4.5 z	11.0±5.1 z		
Mean % weight increase per 14-d interval	46.1±22.0 z	39.2±29.0 z		
Length increase—total period	118.3	103.6	191.4	178.9
Weight increase—total period	181.7	171.0	379.5	347.6
Mean condition factor—start	1.26 z	1.27 z	1.19 z	1.32 y
Mean condition factor—end	1.27 z	1.29 z	1.19 z	1.11 x
Mean dry food conversion[a] per 14-d interval	2.2±0.3 z	2.8±1.4 z		
Mean fresh food conversion[a]	34.2±4.5 z	45.8±22.5 z		
Mean food consumption % body weight/d	88.2±31.8 z	81.5±22.1 z		

[a] Average fresh or dry weight food fed (kg) ÷ average weight gain per individual (kg).

Low Density: Fingerlings

During the low-density, abundant-food phase of the study, diploid fish again grew at a faster rate than triploid fish (Table 1). Diploid fish condition factor did not change significantly during this period. By the end of the study, the condition factor for triploid fish had decreased significantly and was significantly lower than that for diploid fish. The growth rates during this period averaged 5.1 g/d for diploids and 4.6 g/d for triploid fish; these are relatively high growth rates for grass carp of this size. Mean pond temperature and dissolved oxygen concentration were 26.0 ± 2.5°C and 7.2 ± 3.5 mg/L, respectively.

Pool Study: Fingerlings

When diploid and triploid fish were cultured in separate pools with unlimited food, diploid fish once again grew faster (2.4 versus 2.3 g/d: Table 1), although not significantly so ($P > 0.05$). Diploids and triploids did not differ significantly in mean condition factor, food conversion, or food consumption. Mean temperature for both pools was 26.3°C (range, 23.0–28.2°C). Mean dissolved oxygen concentrations were 7.7 ± 0.41 and 7.9 ± 0.33 mg/L in the triploid and diploid fish pools, respectively.

Discussion

In every test situation where diploid and triploid grass carp were grown together, diploid fish demonstrated an overall faster growth rate and a significantly higher condition factor than triploid fish. When fingerling fish were overstocked and underfed, the discrepancy between mean diploid and triploid total lengths was least. The greatest difference between diploid and triploid mean total length occurred when fry were grown at a relatively low density with abundant food (Figure 1). No significant differences in growth, condition, food conversion, or food consumption occurred when diploid and triploid fish were grown independently under the same culture conditions, when food was not limited. The combined effect of a slightly more efficient food conversion and higher consumption rate of the diploid fish is probably responsible for their faster growth when grown with triploid fish competing for the same resources.

Gervai et al. (1980) reported that the growth rate of triploid juvenile common carp (*Cyprinus carpio*) feeding ad libitum was not significantly different from that of diploid fish. A comparison of growth for diploid and triploid threespine sticklebacks (*Gasterosteus aculeatus*) indicated that triploid fish grew no faster than diploid individuals (Swarup 1959). Benfey and Sutterlin (1984) found no significant difference in the weight of diploid and triploid Atlantic salmon (*Salmo salar*) but triploid fish had a lower condition factor. Faster growth has been reported for 8-month-old triploid channel catfish (*Ictalurus punctatus*) and for 14-week-old triploid blue tilapia (*Tilapia aurea*) compared to diploid individuals (Valenti 1975; Wolters et al. 1982).

The faster growth rate of diploid than of triploid grass carp can be useful for distinguishing one group from the other and for reducing the time spent handling and certifying the triploids. With reference to the length-frequency distributions in Figure 1, grading and discarding fish longer than 105 mm would have reduced the number of diploid fish by 63.8% and increased the percentage of triploids remaining from 50 to 71.6%. Also, differences in condition between diploid and triploid fish can be visually detected at total lengths of 300 mm or more. The reliability of sorting diploid from triploid fish based on the visual perception of plumpness was greater than 90% after 10 or 15 min of practice.

A decision to incorporate large-scale grading as a presegregation technique depends on the value of time and labor relative to the value of triploid fish lost in the grading process. We recommend this procedure when the proportion of triploid fish is low (i.e., <50%) or when the number of available triploid fish greatly exceeds the number needed. For example, we would recommend this procedure in a situation where 500,000 larvae will yield 25,000 triploids (5%) but only 15,000 triploids are needed.

In conclusion, growth rate differences in diploid and triploid grass carp fingerlings can be used to presegregate diploid from triploid grass carp when the fish are grown in low-density, abundant-food situations that sustain rapid growth (2–5 g/d). Segregating diploid and triploid grass carp cultured under these conditions when fish have total lengths of 50–140 cm can result in a considerable savings in handling time and elimination of at least 50% of unwanted diploids. Visual sorting of triploid fish, which are less plump, can be accomplished with larger fish (>300 mm total length) with reasonably high accuracy but this procedure is more time consuming because each individual must be examined.

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Third-Year Growth of Channel Catfish

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Abstract.—Fish production, feed consumption, and feed conversion were evaluated for 2-year-old channel catfish (*Ictalurus punctatus*) raised an additional growing season. Channel catfish averaging 1.5 lb each were stocked into two 4.0-acre ponds and fed a floating 32% protein commercial feed for approximately 160 d. At harvest the fish averaged 3.1 lb and attained a feed conversion of 2.2. Feed consumption, expressed as a percent of the estimated standing crop weight, decreased from approximately 1.25 to 0.70% over the growing season.

Most research on production of channel catfish (*Ictalurus punctatus*) has emphasized second-year growth to a market weight of 1.3 lb. Data are limited on fish growth, rates of feed consumption, and feed conversion for larger catfish grown for a third year. Such data would assist culturists in production planning and aid economists in determining production costs for larger fish. Grey (1982) stated that 1.0-lb channel catfish raised at a density of 1,500 fish/acre can attain an average weight of 2.5 lb during a third growing season, but he did not provide supporting data. He recommended a daily feeding rate of 2% of the standing crop weight but he did not discuss feed consumption or feed conversion. Dupree (1984) reported that channel catfish brood stock are normally fed 1–2% of their body weights during warm weather but only 0.5–0.75% in colder periods. He noted that large channel catfish are more wary than smaller fish and are sometimes hesitant to feed near the surface, making it more difficult to feed them a floating fish feed. Lovell (1984) observed that feed consumption, growth rate, and feed conversion generally decrease as fish size increases. He reported that channel catfish initially weighing an average of 1.2 lb consumed feed in a 3-month period (June–August) at a rate of 1.2–1.4% of the estimated standing crop weight and attained a feed conversion of 2.16.

In the present study, I evaluated fish production, feed consumption, and feed conversion for channel catfish raised during a third growing season in west-central Mississippi.

Methods

In April 1983, age-2 channel catfish, average weight 1.5 lb, were stocked at a density of 1,500 fish/acre (2,179 lb/acre) into duplicate 4.0-acre ponds. The fish were fed a commercial 32% protein floating feed once daily, between 1300–1500 hours, 7 d/week. The fish were fed an amount they would consume in 15–20 min based on the feeding response at feeding time. No attempt was made to measure wasted feed; it was assumed that all feed was consumed. Standing crop was estimated at 3-week intervals from fish samples collected by seining. Dissolved oxygen (DO) was measured periodically and paddlewheel aerators were used whenever DO decreased to 2.0 ppm.

Results and Discussion

When harvested in late September and early October 1983, fish standing crops in the two ponds averaged 4,636 lb/acre, fish production averaged 2,452 lb/acre, and average fish weight was 3.1 lb (Table 1). Fish survival was greater than 98% in both ponds. Over 90% of harvested fish attained a minimum size of 1.5 lb and more than 50% weighed at least 3.0 lb (Table 2).

The fish in the two ponds were fed for 156 and 163 days, respectively. Feed conversion (weight of feed fed ÷ fish weight gain) averaged 2.2. Feeding activity varied considerably from day to day throughout the growing season (Table 3). The fish seldom ate more than 60 lb feed per acre per day.

Table 1.—Fish production and feed conversion for 2-year-old channel catfish stocked at the rate of 1,500 fish/acre in ponds and fed a commercial 32% protein floating fish feed during the 1983 growing season.

Pond (feeding period)	Stocking weight (lb/acre)	Harvest weight (lb/acre)	Fish production (lb/acre)	Feed conversion[a]
1 (156 d)	2,154	4,448	2,294	2.2
2 (163 d)	2,205	4,815	2,610	2.2
Average	2,179	4,636	2,452	2.2

[a] Feed conversion = weight of feed fed ÷ fish weight gain.

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TABLE 2.—Size distribution at stocking and at harvest for 2-year-old channel catfish raised at a density of 1,500 fish/acre in ponds during the 1983 growing season. Percentages were determined from samples of 11% and 22% of the fish stocked and harvested, respectively. All values are averages for two study ponds.

Weight group (lb)	Weight distribution: At stocking (%)	Weight distribution: At harvest (%)
<0.75	15.1	0.0
0.75–1.4	60.7	5.9
1.5–2.9	24.2	40.5
3.0–3.9	0.0	34.2
≥4.0	0.0	19.4

Voluntary feed consumption expressed as a percent of the estimated body weight decreased from approximately 1.25% at the start to 0.70% at the end of the growing season (Table 3). The large channel catfish fed cautiously and were often reluctant to feed at the water surface near the pond bank.

The size of the ponds coupled with the effects of wind and the erratic feeding activity of the catfish near the pond edge sometimes made it difficult to gauge feeding response and the proper amount of feed to allocate daily. Small quantities of wasted feed were observed occasionally and underfeeding surely occurred at times. Nonetheless, these conditions simulate those encountered in large commercial catfish ponds and the feed consumption rates obtained in this study approximate those expected when floating feed is given to large fish.

TABLE 3.—Estimated daily feeding rates and feed allotments (averages of two ponds) for 2-year-old channel catfish fed to satiation once daily with a commercial 32% protein floating fish feed.

Period	Average (range) daily feed allotment (lb/acre)	Average daily feeding rate (% of standing crop)
Apr 12–30	26 (25–33)	1.1
May 1–15	35 (13–75)	1.3
May 16–31	37 (13–75)	1.2
Jun 1–15	30 (13–69)	0.9
Jun 16–30	37 (13–75)	1.1
Jul 1–15	44 (25–75)	1.2
Jul 16–31	38 (10–81)	1.0
Aug 1–15	35 (13–63)	0.9
Aug 16–31	28 (6–63)	0.7
Sep 1–15	35 (8–63)	0.8
Sep 16–30	33 (19–63)	0.7

In this study, 2-year-old channel catfish stocked at a density of 1,500 fish/acre (2,179 lb/acre) during their third growing season essentially doubled their total weight with a feed conversion of 2.2. For comparison, Busch (1985) provided data from a separate study, conducted with a similar methodology during the same growing season, for fingerling channel catfish stocked at an average weight of 0.04 lb and raised to an average weight of 1.0 lb. The fingerlings were stocked at densities of 6,000 fish/acre into three 4.0-acre ponds in May 1983 and were harvested in the fall. The standing crops at harvest averaged 5,482 lb/acre, fish production averaged 5,225 lb/acre, and the average feed conversion was 1.6.

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Digestibility in Rainbow Trout: Comparison of the Direct and Indirect Methods of Measurement

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Abstract.—The present study validated a method for the automatic collection of fish feces, by comparing digestibility data obtained by the direct (total feces collection) and indirect (chromic oxide tracer) methods. Six groups of 15 rainbow trout (*Salmo gairdneri*) were successively fed four pelleted diets, three of which contained 30% (dry weight) of chironomid larvae, daphnids, or gammarids. Apparent digestibility coefficients were calculated by the direct and indirect methods for dry matter, crude protein, and energy content. Results were identical with both methods for the daphnid and gammarid diets. The direct method gave significantly higher digestibility coefficients for the reference diet and the chironomid diet. It is possible to obtain reliable digestibility figures by the direct method with an automatic fish feces collector for some types of diet.

One of the elements required for the assessment of fish nutrition is a dependable measurement of the digestibility of the various nutrients. The problem is essentially that of establishing the appropriate balance between the nutrient input from the feed and its output through the feces. Two methods are available. The *direct* method (Tunison et al. 1944; Post et al. 1965; Ogino et al. 1973) consists of quantitatively collecting the feces emitted that correspond to one or several meals. An *indirect* method has been developed to obviate quantitative collection problems by using an inert internal tracer; most frequently, chromic oxide is incorporated into the feed and analyzed both in the feed and in the feces. Digestibility for a given nutrient can then be calculated (Maynard and Loosli 1969).

The most serious difficulty in both methods is the collection of representative fish feces. Various techniques have been proposed and used: abdominal pressure (Nose 1967), anal suction (Windell et al. 1978), dissection (Phillips et al. 1948; Nose 1960; Hastings 1969; Austreng 1978), metabolic chamber (Post et al. 1965), settling of the feces (Cho and Slinger 1979), and continuous filtration of water from the fish tank (Possompes 1973; Kaushik and Luquet 1976; Choubert et al. 1979). All these techniques suffer from one drawback or another, such as collection of representative material (Smith and Lovell 1972; Austreng 1978; Windell et al. 1978) or leaching of nutrients from the feces in contact with water (Inaba et al. 1962; Possompes 1973; Windell et al. 1978). These criticisms prompted Choubert et al. (1982) to improve an automatic fish feces collector designed to rapidly separate the feces from water and, at the same time, to allow quantitative collection of feces over long periods of time.

The incorporation of tracers such as chromic oxide in the feed is also open to criticism, especially when quantitative recovery of the tracer does not occur, a problem demonstrated for fish (Fromm and Stokes 1962; Foltz 1979) and other species (Thill et al. 1978; Guilloteau and Toullec 1980). The direct method for digestibility studies therefore appears to be desirable as a routine methodology. Moreover, the direct method allows the use of live food.

The present work documents the validity of the routine methodology developed by Choubert et al. (1982) by comparing digestibility data obtained by both the direct and indirect methods.

Methods

The experiments were conducted with six lots of 15 rainbow trout (*Salmo gairdneri*; 70 g initial weight randomly distributed) in 60-L cylindroconical tanks. The temperature of the recirculated water was 19 ± 1°C.

The fish were adapted to the tanks for 10 d and to their various diets for 2 d (de la Noüe et al. 1980) before the feces were collected with the automatic collector previously described (Choubert et al. 1982). With this collector, waste water from the tanks flows (4 L/min) through 650-μm-mesh metal screens that separate feces from water as the screens move linearly. The feces are then ejected into pans where they can be frozen within 30 s.

TABLE 1.—Composition of reference and experimental diets (mean ± SD; $N = 4$; WW = wet weight; DW = dry weight).

Diet	Diet constituent: Reference[a] (% of WW)	Diet constituent: Test ingredient (% of WW)	Dry matter (% of WW)	Cr_2O_3 (% of DW)	Crude protein (N×6.25) (% of DW)	Energy (kJ/g DW)
Reference	100	0	92.1±0.3	1.47±0.12	66.7±2.0	19.8±0.4
Reference : chironomid larvae	70	30	91.7±0.4	1.09±0.04	61.5±0.8	19.1±0.4
Reference : daphnids	70	30	93.7±0.6	1.16±0.06	65.8±0.9	20.0±0.8
Reference : gammarids	70	30	91.7±0.6	1.04±0.04	69.9±1.2	19.0±0.4

[a] Fish meal 91.5%; mineral mix (Luquet 1971) 0.97%; vitamin mix (EIFAC 1971) 2.93%; lignosulfite 0.97%; alginate 2.14%. Proportions are given on a dry weight basis.

The time required between emission from the fish and separation from the water does not exceed 17 s.

The diets successively used (Table 1) were a reference diet and three diets incorporating 30% invertebrate biomasses (on a dry weight basis) with the reference diet: (1) reference : chironomid larvae, (2) reference : gammarids, and (3) reference : daphnids. The diets were prepared in the laboratory by mixing the rehydrated ingredients, pelleting the resulting slurry and drying the pellets at 35°C. The chironomid larvae and the gammarids (unknown species) were purchased (Lyard, F73370, Le Bourget du Lac, France; and Gresbil, F95880, Enghien-les-Bains, France, respectively); daphnids (probably *Daphnia* sp.) were provided by Dr. Barnabé (Sète, France). The pelleted food was distributed twice a day (at 0900 hours and 1600 hours) in such a way that all the pellets were ingested by the fish. The distributed food amounted to 1.3% of fish live weight and each group of fish ate the same amount each time.

The frozen collected feces were pooled over 5 d for each tank. They were lyophilized, weighed, and ground. Samples were used for analyses of dry matter (24 h at 95°C), chromic oxide (Bolin et al. 1952), crude protein (N × 6.25; Kjeldahl), and energy content (Parr adiabatic microcalorimeter bomb).

The apparent digestibility coefficient for a given nutrient or energy content according to the indirect method (ADCi) was calculated by the relation of Maynard and Loosli (1969):

$$\text{ADCi} = 100\{1 - [(\text{nutrient in feces}/\text{nutrient in diet}) \times (\%\ Cr_2O_3 \text{ in diet}/\%\ Cr_2O_3 \text{ in feces})]\}.$$

The apparent digestibility coefficient for a given nutrient or energy content according to the direct method (ADCd) was calculated by the formula

$$\text{ADCd} = 100\{1 - [(\text{nutrient in feces} \times \text{feces dry weight})/(\text{nutrient in diet} \times \text{food dry weight})]\}.$$

The statistical analysis (t-tests) of data was according to Snedecor and Cochran (1967).

Results

The diets were quite isonitrogenous and had similar energy contents (Table 1). The incorporation of invertebrate biomasses (especially daphnids and gammarids) resulted in firmer pellets leading to better-formed feces.

Although the quantity of ingested food was the same for the four diets (around 14 g), the quantity of collected feces differed among diets from a low 2.47 g for the reference diet to a high 4.34 g for the reference : gammarid diet (Table 2). Chromic oxide recovery (established by the balance between the food fed with a known chromic oxide content, and the feces collected with a measured chromic oxide content) was not constant amongst the various diets. The reference and the reference : chironomid diets led to an apparent loss of chromium (83.7% and 93.0% recovery of chromium, respectively), whereas recoveries of fecal chromium were 96.3% for the reference : daphnid diet and 102.3% for the reference : gammarid diet. A few feces were lost outside the collecting pans.

The digestibility of dry matter by rainbow trout estimated by the direct and the indirect methods did not differ significantly ($P > 0.05$) for either the reference : daphnid or the reference : gammarid diets (Figure 1). The reference and reference : chironomid diets, however, each gave a significantly higher ($P < 0.01$) digestibility coefficient with the

TABLE 2.—Quantity of food ingested by rainbow trout and quantity and composition of feces collected during digestibility studies (mean ± SD; $N = 6$; all values are on a dry weight basis).

Diet	Food ingested (g/d)	Feces collected (g/d)	Composition of collected feces		
			Cr_2O_3 (%)	Crude protein (N × 6.25) (%)	Energy (kJ/d)
Reference	13.81	2.47±0.08	6.88±0.22	32.2±0.88	9.52±0.61
Reference : chironomid larvae	13.76	3.29±0.12	4.24±0.08	28.8±0.65	11.20±0.27
Reference : daphnids	14.05	3.45±0.12	4.55±0.10	31.9±1.11	11.29±0.24
Reference : gammarids	13.75	4.34±0.13	3.37±0.07	27.8±0.65	10.79±0.29

direct method than with the indirect method. The digestibility of dry matter in the reference diet was always higher than the values measured for the other diets. The maximum difference (14%) occurred between the reference and the reference : gammarid diets.

As for dry matter, digestibility values for crude protein (which exceeded 85%) did not differ significantly for the reference : daphnid and the reference : gammarid diets, but the direct method gave significantly higher values than the indirect method for the reference diet and for the reference : chironomid diet (Figure 2). Only in the case of the reference diet was the ADCd of crude protein higher than 90%. The difference between the highest crude protein digestibility (reference diet) and the lowest (reference : gammarid diet) was about 5%.

Digestibility coefficients of energy showed similar trends (Figure 3). There was no significant difference between the direct and indirect methods for the reference : daphnid and the reference : gammarid diets, but again we observed a significantly higher digestibility coefficient with the direct method than with the indirect method for the reference diet and for the reference : chironomid diet. The difference between the highest (around 90% for the reference diet) and the lowest values (82% for the reference : gammarid diet) was considerable.

Discussion

Three problems arise when an inert marker, such as chromic oxide, is used for digestibility studies of fish. The first one is representative collection of feces. Since the indirect method does not call for a total quantitative recovery of feces, it is necessary to assume that the quality of feces collected (Furukawa 1973) does not change with time or with the nycthemeral cycle (Possompes 1973). This difficulty is further increased by the possibility that nutrients will be leached from the feces once the feces have been emitted. The third difficulty arises

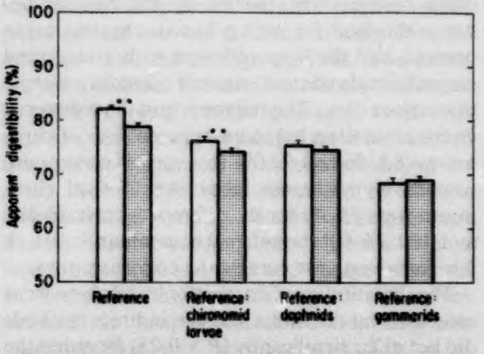

FIGURE 1.—Apparent digestibilities of dry matter by rainbow trout fed four diets, according to the direct (unshaded) and indirect (shaded) methods (mean ± SE; $N = 6$). Asterisks denote significant differences between paired means (t-tests; $P \leq 0.01$**).

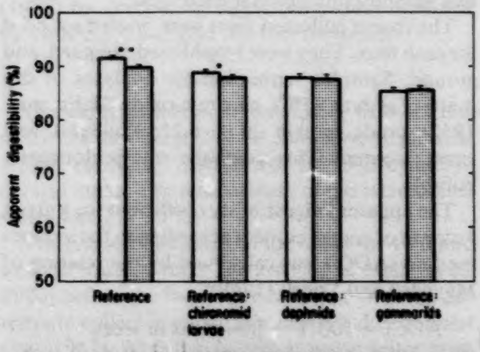

FIGURE 2.—Apparent digestibilities of crude protein by rainbow trout fed four diets, according to the direct (unshaded) and indirect (shaded) methods (mean ± SE; $N = 6$). Asterisks denote significant differences between paired means (t-tests; $P \leq 0.05$* or $P \leq 0.01$**).

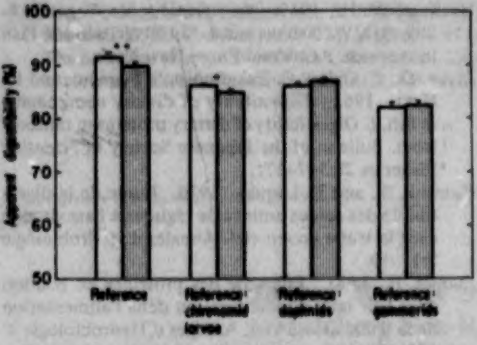

FIGURE 3.—Apparent digestibility of energy by rainbow trout fed four diets, according to the direct (unshaded) and indirect (shaded) methods (mean ± SE; N = 6). Asterisks denote significant differences between paired means (t-tests; $P \leq 0.05^*$ or $P \leq 0.01^{**}$).

when it is difficult, undesirable, or impossible to incorporate an inert marker in the diet. This is clearly the case with live food, for which one is restricted to the use of natural indigestible markers, when available.

To be useful, the direct method requires the total collection of feces. The problem is not only the quantitative collection of the feces, but also dissolution of feces. It has been shown (Inaba et al. 1962; Possompes 1973; Windell et al. 1978) that dissolution of feces starts rapidly in water and this leads to an underestimation of 10–15% of the measured nitrogen in feces and, therefore, to an overestimation of digestibility coefficients (Possompes 1973; Smith 1976; Windell et al. 1978; Smith et al. 1980).

In the present study, the feces were separated from water within 6–17 s, reducing leaching problems to a minimum. Demonstrating such a point is difficult; collecting the feces directly from the fish for comparison with feces spontaneously emitted in water is open to criticism since the fish must be either stressed or anesthetized. If leaching of feces occurs within 10 min (Possompes 1973), it is reasonable to propose that the short collection time measured in the present work precludes any important leaching of the feces.

The nature of the food supply might be important for the interpretation of some results. It is apparent (Tables 1, 2) that the use of diets incorporating biomasses such as daphnids or gammarids results in a nearly complete recovery of distributed (and ingested) chromic oxide. This is not the case for the reference (fish-meal) and reference : chironomid diets. With the latter diets, it was obvious that the pellets and the feces were more fragile and there was some loss of material (chromic oxide and dry matter) during the collection of the feces. This would explain why, in the reference and reference : chironomid diets, the digestibility coefficients obtained by the direct method were consistently higher for dry matter, crude protein, and energy as compared to those measured by the indirect method. Since the same collected feces were used for the calculation of ADCi and ADCd, these results favor a selective loss of chromic oxide. A loss of 10% of fecal chromic oxide would lead to an ADCi about 2% lower than the corresponding ADCd. A loss of 10% appears to be a maximum. The observed loss of fecal material was marginal and its effect would be to slightly increase both the ADCi and ADCd coefficients.

The automatic collector for fish feces (Choubert et al. 1982) answers the various criticisms made of other methods for collecting fish feces. The results obtained in the present study support the conclusion that a quantitative collection of feces can be achieved and that the dissolution of fecal nutrients is reduced to a minimum. This is documented by the comparison of the results for digestibility (Figures 1–3), which are identical for the indirect and the direct methods of measuring digestibility of the reference : daphnid and the reference : gammarid diets. The present results are the first systematic comparisons between direct and indirect methods made with the automatic fish feces collector.

The validity of the methodology used in the present study is apparent when one considers the small dispersion of the values around the means. We conclude that this methodology is valuable for determinations by the indirect method. It can be used for the direct method, too, provided that the food pellets are solid and that the feces are well formed. This can be accomplished by slightly increasing the binding agent concentration in the diet. The small loss of feces observed with some diets calls for an improvement of the collecting device. The most apparent problem is the distance between the collecting grid and the pan into which the feces are propelled. This results in loss of ill-formed feces and fecal fragments. Reduction in the mesh size of the screens does not appear desirable. Although it might allow the recovery of small fragments likely to be lost with the present mesh size, preliminary trials with a finer mesh have shown that much more water is collected, requiring a decreased collection time due to limited pan capacity.

Acknowledgments

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Effect of Release Size on Return Rates of Hatchery Sea-Run Cutthroat Trout

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Abstract.—Optimum release size for Cowlitz, Washington, hatchery sea-run cutthroat trout (*Salmo clarki*) was determined for the 1982 and 1983 year classes by tagging smolts at release. Hatchery returns and sportfishing harvest indicated fish released when they were 21 cm in fork length or larger averaged 12.8% return compared to 2.3% return for smolts less than 21 cm. Based on returns, initial migrant maturity was about 85% for males and 69% for females. Sportfishing harvest accounted for 23.5 and 48.6% of tag returns for each year class, respectively. Nonmigrating cutthroat trout were significantly smaller than smolts and were predominately females.

Smolt size at release can affect survival of hatchery salmonids (Hager and Noble 1976; Washington 1982). An optimum smolt size probably exists for each species; fish reared to a larger size result in excessive costs without commensurate survival improvement. The Cowlitz trout hatchery in Washington state has attempted to plant about 100,000 sea-run cutthroat trout (*Salmo clarki*) smolts each year since 1967. Information on optimum smolt size was lacking for these fish and an experiment was conducted to determine this value. Information on maturity, nonmigrating fish, and harvest rates also was collected.

Sea-run cutthroat trout are thought to return to fresh water regardless of maturity in the first fall after spring emigration. Those not mature upon their initial return overwinter in fresh water, migrate to the estuary or saltwater in spring, and mature as repeat migrants the following fall (Giger 1972). Low rates of maturity among cutthroat trout upon their initial return have been a conservation concern because immature fish have to survive a sport fishery for 2 years prior to spawning (Johnston 1979).

Methods

In 1982, 200 smolts in each 1-cm fork length interval from 16 to 25 cm were tagged at release at the Cowlitz trout hatchery with small (3.5 cm) numbered Floy anchor tags. In 1983, 175 smolts in each 1-cm length interval from 18 to 23 cm were tagged at release. Smolts were released each year into the Cowlitz River in mid-April. Tags were recovered from fish returning to the hatchery and from fish taken in the sport harvest over the following 2 years. Tag recovery boxes were set up near popular angling locations and newspapers were contacted to encourage anglers to return tags. Anglers also were contacted during occasional creel checks.

Mean length of released smolts was determined in 1981 through 1984 and compared with nonmigrant fish. A minimum size criterion of 16 cm for smolt and nonmigrant cutthroat trout was used in 1981 and 1982; 18 cm was used in 1983 and 1984. Nonmigrants were sampled by hook and line for length measurements. Nonmigrants and samples of smolts were killed in 1983 and 1984 to determine sex ratios. Student's *t*-test was used to compare lengths of fish and chi-square was used to test significance of male : female ratios.

Results

Adult Returns

Sportfishing and hatchery returns of the tagged 1982 release indicated larger smolts survived significantly better ($P = 0.05$); there was an average 13.7% return of smolts 21 cm and larger compared to 2.4% for smaller smolts (Figure 1). Only three tagged fish smaller than 18 cm were observed; these fish were nonmigrants captured near the release

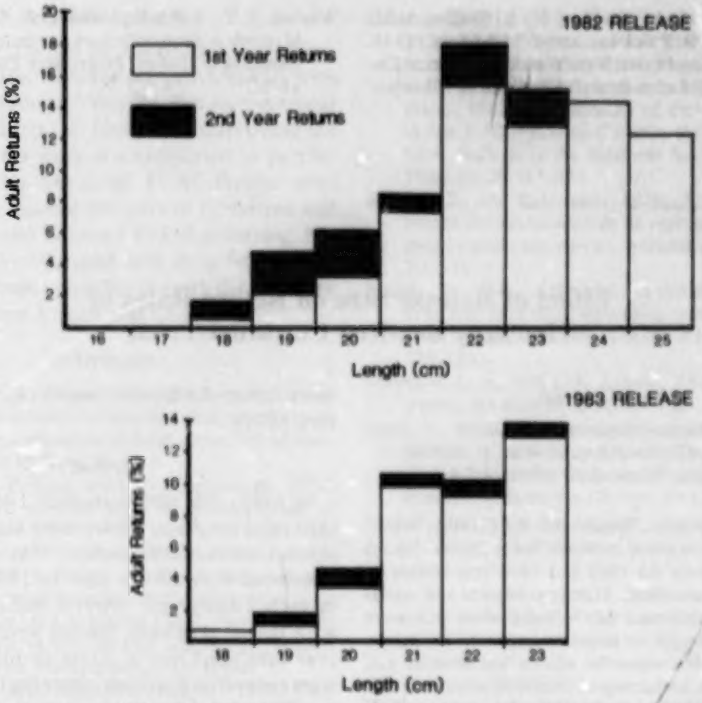

FIGURE 1.—Adult returns of Cowlitz, Washington, hatchery cutthroat trout in relation to size (fork length) at release.

site several months after release. Among recaptured fish from the 1982 release, 85.2% returned or were caught in 1982 and 14.8% were recovered the following year. A large percentage of smolts 20 cm and less returned in their second year.

Returns from the 1983 release showed similar survival advantages for large smolts (Figure 1). Smolts 21 cm and larger had an average return of 11.8% while smaller smolts had a 2.3% return. Of the total return from the 1983 release, 87.8% were caught or returned to the hatchery in 1983 and 12.2% were recovered the following year. Returns in the second year did not appear to favor the smaller size groups.

TABLE 1.—Percent maturity of returning Cowlitz, Washington, hatchery sea-run cutthroat trout, based on hatchery returns.

	First return migration		Second return migration	
Year released	Males (%)	Females (%)	Males (%)	Females (%)
1981	85.2	63.6	14.8	36.4
1982	87.1	74.0	12.9	26.0
1983	82.1	70.0	17.9	30.0

Maturity

Based on returns of tagged cutthroat trout to the hatchery, about 85% of the males and 69% of the females matured on the initial return migration (Table 1). Included are data from 1981 tagged and released smolts that were 20+ cm long.

Sportfishing Harvest

Sportfishing accounted for 23.5% and 48.6% of returns from the 1982 and 1983 releases, respectively; 21.1% and 2.8% of the harvest occurred in the second year after release for 1982 and 1983, respectively. An average of 65.8% of tagged fish caught were caught on the Cowlitz River, 31.5% on the Columbia River, and 2.7% elsewhere. Sportfishing harvest figures are minimum values because not all anglers returned tags.

Nonmigrants

Mean length of nonmigrants was significantly ($P = 0.05$) smaller than that of smolts in every

TABLE 2.—Mean fork lengths of, and % females among, cutthroat trout smolts and nonmigrants released from the Cowlitz, Washington, hatchery.

Year	Mean length, cm ± SD (*N*)		% females (*N*)	
	Smolts	Nonmigrants	Smolts	Nonmigrants
1981	20.3 ± 2.3 (321)	19.7 ± 2.7 (126)		
1982	21.7 ± 2.4 (1,117)	18.9 ± 2.5 (210)		
1983	20.4 ± 1.5 (676)	19.8 ± 1.4 (73)	36.9 (111)	67.1 (73)
1984	21.3 ± 1.7 (320)	19.4 ± 1.0 (48)	45.9 (85)	60.4 (48)

year examined (Table 2). The majority of nonmigrants were females and a majority of smolts were males; in 1983, these majorities were significant ($P = 0.05$).

Discussion

Optimum smolt release size appears to be about 22 cm for Cowlitz River sea-run cutthroat trout. Fish smaller than this had reduced survival, and nonmigration probably was a major cause. Size-related tendencies toward nonmigration were noted by Royal (1972) for steelhead (*Salmo gairdneri*).

Differences in mean size of male and female smolts could explain why the Cowlitz hatchery run is dominated by males at a ratio of about 3:1. Washington (1982) observed a size difference between male and female smolts of coho salmon (*Oncorhynchus kisutch*). Because small Cowlitz cutthroat trout smolts tend not to migrate and most nonmigrants are females, a predominance of male adults would be expected. If the hatchery release size is targeted at 22+ cm, females should make up a larger component of returning fish.

Maturity of cutthroat trout upon their initial return migration was greater than expected. Johnston (1979) felt maturity of wild sea-run cutthroat trout was about 14% for first-return females in the Puget Sound area of Washington and, subsequently, restrictive angling regulations were imposed. If maturity of the Cowlitz hatchery stock of cutthroat trout reflects other fish stocks throughout the Columbia River system, angling restrictions to allow females to survive until sexually mature may not be needed. However, a minimum sportfishing harvest rate of 49% of a year class could be cause for concern because few adults would remain to reproduce. With increased run sizes resulting from increased smolt survival, angling regulations have been liberalized in recent years for Cowlitz River cutthroat trout.

There is a possibility of tags causing some size-related mortality. However, Eames and Hino (1983) found similar survival among juvenile 15-cm-long chinook salmon (*Oncorhynchus tshawytscha*) whether they were marked with anchor tags or coded wires or were untagged with coded wires.

A large portion of cutthroat trout smolts released from the Cowlitz hatchery have historically been less than 20 cm, thus their survival has probably been suboptimal. Efforts are presently being made to increase smolt size at the hatchery.

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Seasonal Variation in Channel Catfish Virus Antibody Titers in Adult Channel Catfish[1]

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Abstract.—Serum antibody titers for channel catfish virus (CCV) were measured in adult channel catfish (*Ictalurus punctatus*) that had survived an epizootic of channel catfish virus disease (CCVD) as fingerlings. Serum antibody titer data were grouped into four categories for statistical analysis based on the following titer levels: <1, no titer; 1–10, low; 10–30, medium; and >30, high. Significantly lower antibody titers were observed in January and April than in July and October. The increase in the number of high titers concurrent with elevated water temperature may indicate the existence of carriers persisting 4 ye[illegible]ter the original epizootic. Based on the findings [illegible] study, success in isolating CCV from channel catf[illegible]uring colder months of the year may be enhanced, in part, by the low immune status of the fish at this time.

Channel catfish virus disease (CCVD) is a herpesvirus infection that can cause serious losses of cultured fry and fingerling channel catfish (*Ictalurus punctatus*) (Plumb 1977). Adult channel catfish are a potential reservoir for the virus. Circumstantial evidence of such a carrier state in adult fish is supported by the presence of serum antibodies against channel catfish virus (CCV) in adult fish that have been associated with CCVD epizootics. The purpose of this study was to determine if there is a seasonal variation in serum antibody titers of adult fish that survived a CCVD epizootic as fingerlings.

Methods

Channel catfish used in this study were selected from the survivors of a CCVD epizootic in July 1980. The epizootic resulted in the loss of approximately 140,000 fingerlings from a pond-held population of 200,000. A portion of the survivors were maintained in our laboratory until the study period began in October 1983. The fish were held in 1,900-L circular fiberglass tanks supplied with dechlorinated municipal water at approximately 2 L/min. A commercial sinking catfish pellet was provided at a daily rate of 2% of body weight. During the study period, fish ranged in size from 1 to 2 kg.

From October 1983 to July 1984, 60 fish, randomly selected from a population of 120 fish, were sampled at 3-month intervals. Approximately 2 mL of blood was collected from the caudal artery of each fish into nonheparinized evacuated blood-collecting tubes. Blood was allowed to clot and the serum was collected and stored at −80°C until processed.

Serum antibody titers were measured by a constant virus–varying serum method (Plumb and Bowser 1983). Twofold serum dilutions from 1:5 to 1:320 were incubated for 1 h at 30°C with an equal volume containing 100 tissue-culture-infective-doses, 50%-endpoint (TCID50) per milliliter of CCV (S80-116), the virus isolate from the 1980 epizootic. Triplicate wells of a 96-well microculture plate were inoculated with 0.1 mL of the virus–serum mixture followed by 0.1 mL of a suspension of channel catfish ovary cells (Bowser and Plumb 1980). Plates were incubated at 30°C for 5 d and then scored for cytopathic effect. Fifty-percent-endpoint, serum-neutralization titers were calculated according to the method of Reed and Muench (1938).

Serum antibody titer data were grouped into four categories before statistical analysis, based on the following titer cutpoints: <1, no titer; 1–10, low; 10–30, medium; >30, high. Significant differences ($P < 0.01$) in distribution of titers between sampling periods were determined from frequency tables in the BMDP Statistical Software Program (Brown 1981).

Results and Discussion

Distributions of antibody titers in January and April were significantly lower than those in October and July (Table 1). No significant differences ($P > 0.01$) in distribution of antibody titers were observed between the months of October and July

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TABLE 1.—Channel catfish virus (CCV) serum-neutralization titers of fish that survived a CCV epizootic as fingerlings in July 1980. Months not sharing the same letter have titer distributions that are significantly different from each other ($P < 0.01$).

Year and month	Temperature (°C)	N	Titer level: No titer <1	Low 1–10	Medium 10–30	High >30
1983						
Oct z	22	60	23	6	12	19
1984						
Jan y	17	60	20	27	11	2
Apr y	20.5	59	27	20	9	3
Jul z	25	59	13	16	9	21

or between the months of January and April. The enhanced ability of fish to develop an immune response at higher temperatures, such as occurred between July and October in our study, is well documented (Anderson 1974). The increase in the number of high titers in July and October may indicate the existence of a carrier state persisting 4 years after the original epizootic concurrent with increasing water temperature.

Bowser et al. (1985) recently isolated CCV from asymptomatic broodfish during the winter. Based on the findings of the current study, their success in isolating the virus may have been due in part to a low immune status of the fish during the colder months of the year. The effect of the immune response on the ability to isolate CCV from a carrier state may warrant further investigation.

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Corneal Cloudiness in Transported Largemouth Bass

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Abstract.—Corneal cloudiness, ranging from barely detectable to complete opaqueness, and corneal perforation were repeatedly observed over several years in largemouth bass (*Micropterus salmoides*) subjected to handling stress. The incidence of corneal clouding and the recovery rate were studied by subjecting largemouth bass (15 cm average total length) to simulated truck transport for 6 to 28 h. Groups of 100 fish were removed from the simulator at about 6-h intervals. Their eyes were examined and pectoral fins adjacent to affected eyes were clipped. The fish were placed in recovery tanks and were examined periodically for 28 d. The incidence of cloudiness after various durations of simulated transport ranged from 46 to 83%. The eyes generally cleared once the fish were removed from the simulator; 80% of the cloudy eyes cleared within 20 to 36 h, and all nonperforated eyes were clear within 7 d.

During 1979–1981, the staff of the National Fish Hatchery and Technology Center (NFHTC) repeatedly noticed that up to 50% of advanced fingerling largemouth bass (*Micropterus salmoides*) developed eye abnormalities after they were handled. Slight to total opaqueness of the cornea and, in some cases, even corneal perforation were observed.

Nutritional problems were suspected because various eye abnormalities in fish have been associated with diet deficiencies or imbalances in amino acids (Poston et al. 1977; Rumsey et al. 1983), in vitamins (Poston et al. 1977; Hughes et al. 1981a, 1981b), or in minerals (Poston et al. 1977; Ketola 1979). Several nonnutritional factors were also considered; these included light intensity (Steucke et al. 1968), exposure to heavy metals (Blaxter 1977; Bodammer 1985), and abrasion from rubbing against screens, nets, raceway walls, or other fish (Nicol 1981).

During the course of stress-related studies (Carmichael et al. 1984), it was observed but not reported that cloudy eyes could be induced by subjecting largemouth bass to simulated long-distance truck transport (G. J. Carmichael, personal communication). Further, there was a relationship between the severity of eye abnormality and the intensity and duration of the stress imposed on the fish.

The implications of those preliminary findings were of concern because largemouth bass are often subjected to extended transport under stressful conditions. Stocking fish directly into natural waters after lengthy transport might be a questionable practice, for if handling stress reduces their vision it could increase their vulnerability to predation and affect their ability to capture prey. We attempted to determine if the stress associated with fish transportation could induce corneal changes and, if it did, to determine if the corneal changes were related to the length and severity of the stress and if the changes were reversible.

Methods

During May 1982, fingerling largemouth bass were obtained from the Inks Dam National Fish Hatchery, Burnet, Texas. The fish were fed pelleted feeds until September 1982, at which time about 1,700 largemouth bass (averaging 15 cm total length) were stocked into seven 950-L fiberglass tanks supplied with 22°C well water. The fish were fed for 5 d and then fasted for 48 h before they were transferred to a fish transport simulator. The simulator was a wooden unit consisting of two compartments. Each compartment had a capacity of approximately 270 L and was fitted with a Frigid Unit (Frigid Units, Incorporated, Toledo, Ohio) to control water temperature and circulation. Oxygen diffusers were used to control dissolved oxygen concentrations.

The compartments, designated A and B, were each filled with 170 L of water to which 30.7 kg of fish (approximately 725 fish) were added. The resulting hauling density of 180 g fish/L water had previously been found at the NFHTC to induce corneal abnormalities. Attempts were made to keep oxygen near saturation and the water temperature ranged between 21 and 24°C. Six hours after fish had been in the simulator, 100 fish (about 4.2 kg) were removed from compartment A. The eyes of each fish were visually examined for corneal abnormalities. When an abnormality was found, the pectoral fin adjacent to the affected eye was clipped. After examination, the fish were transferred to a 950-L circular fiberglass tank. We maintained a hauling density of about 180 g/L by removing 17 L of water from compartment A after the fish had been removed.

Twelve hours after fish were loaded, a second 100-fish sample from compartment A was examined, fin-clipped, and stocked in a second 950-L fiberglass tank. This procedure was continued with fish from compartment B, which were sampled after 18, 24, and 28 h of simulated transport. Each time fish were removed, oxygen and temperature readings were obtained. After test fish were loaded into the simulator, 100 control fish were checked for eye abnormalities, placed in a separate 950-L fiberglass tank, and reexamined for eye abnormalities 24 h later. Eyes of all test fish were examined periodically for 28 d after the fish had been placed in the simulator. During the recovery period, fish were fed pelleted feed 5 d/week.

Results and Discussion

Water temperature fluctuated between 15 and 21°C in [illegible]mpartment A and between 22 and 24°C in con[illegible]rtment B during the simulated transport. Oxygen concentrations exceeded 10 mg/L in compartment A and 8 mg/L in compartment B. The eyes of all the control fish were clear and apparently normal at both the initial and the 24-h examinations. Two fish in compartment B died 18 h after initiation of the simulated transport.

Conditions during this study adversely affected 70% of the fish. The length of time fish were stressed was generally related to the percentage of corneas adversely affected (Table 1). The higher frequency

TABLE 1.—Percentage of largemouth bass with corneal abnormalities after varying periods of stress from simulated transport.

Time in simulator	Right eye only (%)	Left eye only (%)	Both eyes (%)	Total (%)
0	0	0	0	0
6	21	10	43	74
12	22	15	9	46
18	19	15	35	69
24	24	11	43	78
28	16	20	47	83

of eye abnormalities observed after 6 h of simulated transport (74%) than after 12 h (46%) is unexplained. We cannot explain the apparently random unilateral or bilateral appearance of the corneal abnormalities on individual fish. We made no quantitative assessment of degree of opaqueness. However, on a qualitative basis, it appeared obvious to us that opacity increased as the length of the simulated transport increased. During previous transport hauls from the NFHTC involving more than 10,000 largemouth bass, fish were observed with corneas that were perforated or had become so weakened that merely touching the area near the eye resulted in the lens tearing through the cornea. Mortality among such fish was high. This condition was not observed during the present study. Overall mortality in this study was less than 1% during the simulated transport and recovery periods.

During the recovery period all eyes were examined to determine the recovery rate and to see if corneal clouding continued to develop after the transport stress was removed. Among the 1,000 eyes examined (500 fish), 473 had cloudy corneas; of these 473, only 24 had not had abnormalities when the fish were removed from the simulator and fin-clipped. It thus appears that, once the stress is removed, the possibility of a clear cornea becoming cloudy is low (less than 3% in this study). It also appears that the corneal cloudiness is reversible. More than 75% of all fish had recovered within 48 h after the stress was removed and 100% recovered within 7 d.

It has been found that transportation does stress largemouth bass and that the effects of transportation can be partially alleviated by adding salts to the hauling water and following prescribed procedures (Carmichael et al. 1984). In this study, salts were not used. We believe that largemouth bass should be examined after they are transported. If their eyes are found to be impaired, a decision must be made concerning the stocking of the fish. Corneal clouding is believed to impair vision and, when severe enough, it may effect the ability of the fish to perceive and escape a predator. If possible, the fish should be held until their corneas have cleared, although we realize that this holding period is not always feasible.

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Effect of Food Concentration on the Survival and Growth of Australian Bass (*Macquaria novemaculeata*) Larvae

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Abstract.—Larvae of Australian bass (*Macquaria novemaculeata*) were offered various concentrations of rotifers (*Brachionus plicatilis*) at 0–24 organisms/mL for 2 weeks starting at completion of yolk absorption. In another experiment, larvae initially fed rotifers for 2 weeks were offered brine shrimp (*Artemia salina*) at concentrations of 0–15 nauplii/mL. In each experiment the concentration of zooplankton had a significant ($P < 0.01$) effect on larval survival. The minimum concentrations that resulted in high survival and growth rates of larvae were 9 rotifers/mL for the first 2 weeks after yolk absorption and 6 brine shrimp nauplii/mL for the next 2 weeks.

The Australian bass (*Macquaria novemaculeata*) is a native percichthyid fish of southeastern Australia that has potential for aquaculture (van der Wal, personal observation, 1983). Research has been conducted at the Brackish Water Fish Culture Research Station, New South Wales, Australia, to develop reliable techniques for the culture of this catadromous species. The pelagic eggs and larvae develop in brackish water until metamorphosis, after which migration upstream takes place (Williams 1966; Chamberlain, unpublished data, 1976). Larvae of marine and estuarine fishes are difficult to rear because they are small and require live food of suitable size when exogenous feeding begins (Nash and Kuo 1975). However, food concentrations need to be controlled (O'Connell and Raymond 1970; Nash and Shehadeh 1980) and must be relatively high (>1 organism/mL) to achieve good survival and growth (May 1974). Fertilized Australian bass eggs are 1.1 mm in diameter and newly hatched larvae average 3.5 mm long. Inert food has not been successfully fed to Australian bass larvae (Nell, personal observation, 1984).

Rotifers (*Brachionus plicatilis*) and nauplii of brine shrimp (*Artemia salina*) are adequate food items for some marine fish larvae (Watanabe et al. 1983). In the current study, experiments were conducted to determine the concentrations of *Brachionus plicatilis* and *Artemia salina* that produce maximum survival and growth of larvae during the 4 weeks following the completion of yolk absorption.

Methods

Adult Australian bass captured from the wild with gill nets were held in 100,000-L round ponds for at least 12 months prior to the experiments. The fish were kept in fresh water until the start of the natural breeding season in winter and early spring (June–October). During the breeding season they were kept at 28‰ salinity while ovarian maturation took place. Three pairs of fish were induced to spawn by injections of human chorionic gonadotrophin at 1,000 international units/kg. After injection, each pair of fish was kept in 2,000-L circular tanks for spawning. The resulting fertilized eggs were collected from spawning tanks and hatched in 600-L conical tanks with a flow-through water system.

Brachionus plicatilis were cultured on marine algae (*Stichococcus* sp.). The dry weight of the harvested rotifers was 0.26 μg/organism. The *Artemia salina* used (Biological Marine Incorporated, Hawthorne, California) had a dry weight of 2.42 μg/nauplii (Sorgeloos et al. 1983).

The experiments were carried out in 40-L polyethylene aquaria maintained at 20 ± 1°C. there were four randomly allocated aquaria, each containing 100 randomly selected larvae for each live-food concentration. Filtered seawater with salinities ranging from 28 to 34‰ was allowed to flow through the aquaria at the rate of 120 L/d. The live-food organisms and Australian bass larvae were retained in the aquaria by means of suitably sized screens on the outlets. The concentration of live-food organisms in each aquarium was checked daily by counting the numbers in 50-mL aliquots taken from each aquarium. The required number of food organisms was added to make up any shortfalls. At the end of each 2-week experimental period, the number of live postlarvae in each aquarium was recorded and the lengths of 10 larvae were measured.

The data were statistically evaluated by analysis of variance (Steel and Torrie 1960) and least significant differences were calculated to compare mean values.

Experiment 1.—Experiment 1 was designed to determine the optimum concentration of rotifers to feed Australian bass larvae immediately after yolk absorption. The live-food concentrations were 0, 3, 6, 9, 12, 15, 18, 21, and 24 rotifers/mL. Three days after hatching, random samples of 100 larvae were collected and placed into each of 36 aquaria. At the start of the experiment, larvae ranged from 4.1 to 4.3 mm total length. The experiment was completed 2 weeks after yolk absorption.

Experiment 2.—Larvae used in experiment 2 had been reared for 2 weeks past yolk absorption in aquaria containing 9 rotifers/mL. Live-food concentrations were 0, 3, 6, 9, 12, and 15 recently hatched brine shrimp nauplii per milliliter. At the start of the experiment the larvae ranged from 5.8 to 6.3 mm total length.

Results

Experiment 1

All unfed larvae were dead after 7 d. The mean survival rates ranged from 20 to 83% at live-food concentrations of 3 to 9 rotifers/mL and the highest mean survival rate was 85% at a food concentration of 15 rotifers/mL (Table 1). There were no significant ($P > 0.01$) differences between survival rates at concentrations of 9, 12, and 15 rotifers/mL, but all of these were significantly ($P < 0.01$) higher than survival rates at the higher food concentrations of 18 to 24 rotifers/mL (Table 1). The mean lengths of larvae offered 3, 18, or 21 rotifers/mL were significantly ($P < 0.01$) lower than the mean lengths of larvae offered 9, 12, or 15 rotifers/mL (Table 1).

TABLE 1.—Effect of the concentration of rotifers (*Brachionus plicatilis*) on survival and growth of Australian bass larvae (experiment 1). Feeding commenced at completion of yolk absorption and continued for 2 weeks. Values are means ± SD. Within each column, means which do not share a common letter differ significantly ($P < 0.01$).

Number of rotifers/mL	Survival rate (%)	Length (mm)
0	0	
3	20±6.8 x	5.0±0.24 w
6	37±7.7 y	5.6±0.21 yx
9	83±7.4 z	6.1±0.10 z
12	84±6.4 z	5.9±0.08 zy
15	85±4.2 z	5.9±0.15 zy
18	62±9.9 y	5.5±0.10 x
21	58±4.0 y	5.5±0.13 x
24	58±7.8 y	5.6±0.17 yx

TABLE 2.—Effect of the concentration of brine shrimp nauplii (*Artemia salina*) on survival and growth of Australian bass larvae prior to metamorphosis (experiment 2). Feeding commenced 2 weeks after completion of yolk absorption and continued for 2 weeks. Values are means ± SD. Within each column, means which do not share a common letter differ significantly ($P < 0.01$).

Number of *Artemia* nauplii/mL	Survival rate (%)	Length (mm)
0	0	
3	28±2.4 y	8.5±0.26 y
6	78±2.6 z	10.8±0.17 z
9	76±4.2 z	9.6±0.26 zy
12	71±8.7 z	8.4±0.17 y
15	74±4.7 z	8.8±0.17 zy

Experiment 2

All unfed larvae were dead within 2 weeks. The mean survival rates ranged from 28 to 78% at live-food concentrations of 3 and 6 nauplii/mL, respectively (Table 2). There were no significant ($P > 0.01$) differences in the survival rates for concentrations of 6 to 15 nauplii/mL. The mean length increase was highest at a concentration of 6 nauplii/mL (Table 2).

Discussion

Hjort (1914) hypothesized that there was a "critical period" during which lack or shortage of food would be detrimental to fish larvae. For many species this critical period occurs very shortly after the yolk has been completely absorbed. This would be particularly true for larvae hatching from pelagic eggs (Lasker et al. 1970; May 1975), such as Australian bass larvae. In this study, unfed larvae died within 7 d of yolk absorption at a water temperature of 20 ± 1°C. Even though the "point of no return" (May 1974) was not determined in this study, the need for food soon after the completion of yolk absorption was clearly demonstrated.

The highest survival and growth rates occurred when Australian bass larvae were offered food concentrations of 9 to 15 rotifers/mL. The reduced survival rates at 3 and 6 rotifers/mL were probably caused by an insufficient food supply. Small, poorly developed larvae such as these require relatively high concentrations of suitably sized live food organisms at initial feeding (O'Connell and Raymond 1970; Nash and Shehadeh 1980). However, the low survival rates at concentrations of 18 to 24 rotifers/mL appeared to have been due to overfeeding. Spectorova et al. (1974) found that, at high concentrations of rotifers, many organisms pass through the gut of fish larvae undigested. As there was a continuous flow (120 L/d) of water through the aquaria, deterioration of water quality would not have been an important factor. Nash and Kuo (1975) found that domination by an abundance of food organisms appeared to intimidate fish larvae to a stage where reduced feeding resulted. The problem was not so apparent for our older post-yolk-sac larvae, although there was a reduction in growth at food concentrations in excess of 6 brine shrimp nauplii/mL.

Australian bass larvae can be transferred to fresh water 4 weeks after the completion of yolk absorption, when metamorphosis has taken place (van der Wai, unpublished data, 1984). Hence the need for saltwater food organisms exists only during the first 4 weeks of exogenous feeding.

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Zinc Supplementation of Trout Diets: Tissue Indicators of Body Zinc Status

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Abstract.—Rainbow trout (*Salmo gairdneri*) were fed one of three different diets, based either on fish meal, soybean meal, or egg white, containing varying levels of zinc. Several fish tissues were analyzed for zinc content after 202 d to determine if any tissue was a sensitive indicator of zinc status. Caudal fin and whole-body zinc levels of the fish were found to reflect the dietary levels of zinc. We observed a strong statistical correlation between whole-body zinc levels and the zinc concentration of the caudal fin ($r = 0.89$; $P < 0.01$). At the same time, we observed a negative correlation between whole-body iron concentration and whole-body zinc levels ($r = 0.87$; $P < 0.01$). A strong negative correlation also was observed between dietary zinc and whole-body iron ($r = 0.95$; $P < 0.001$). Our data indicate that caudal fin zinc concentrations are a good indicator of zinc status.

In studies of trace metal requirements and metabolism, indicators of current and long-term statuses of specific trace metals are needed (Hamilton 1981). Furthermore, the application of such methods outside the laboratory is useful for diagnosing disease conditions related to trace metal deficiencies. A variety of indicators have been used to measure zinc status in mammals such as hair levels (O'Leary et al. 1980), enzyme activities (Kfoury et al. 1968; Huber and Gershoff 1973), serum levels (Turner et al. 1978), and bone levels (Huber and Gershoff 1970).

Recently, studies of fish have indicated that a wide range of dietary ingredients have the potential to depress whole-body or tissue zinc concentrations, which can eventually lead to growth retardation or physiological disfunctions such as cataracts (Ketola 1979; Hardy and Shearer 1985; Richardson et al. 1985). In these and other studies of the role of dietary zinc in fishes (Ogino and Yang 1978; Watanabe et al. 1980; Jeng and Sun 1981), a variety of organs and tissues have been used as indicators of zinc status. In these studies, however, the sampling methodology required the destruction of the fish. Under some conditions, it is not practical or desirable to sacrifice the animal in order to determine the elemental status. There is a need for a simple, nondestructive method that can be used to quickly assess the zinc status of fish.

We undertook this study to evaluate how accurately the zinc concentrations of various tissues and organs of rainbow trout (*Salmo gairdneri*) reflect dietary zinc levels. We report here a correlation between the zinc concentration in the whole body and in the caudal fin of fish maintained on different diets. We also observed an inverse correlation between whole-body iron and zinc.

Methods

Diet formulation.—The eight experimental diets used in this study were based on three protein sources: fish meal, soybean meal, and egg white (Table 1). Egg-white diets were included because of their inherent low zinc content (4 μg/g), making it possible to use a zinc-deficient diet. Soybean-meal diets were chosen because they bind cations such as iron and zinc (Kratzer et al. 1959; O'Dell et al. 1972). Furthermore, soybean meal is widely used in the formulation of fish diets. A fish-meal diet was included as an internal standard.

Commercial soybean meal and egg white were autoclaved with steam at a pressure of 1.06 kg/cm^2 for 30 min before being added to the diets to reduce residual trypsin inhibitor activity in the soybean meal and aviden in the egg white. Tuna viscera were added to the soybean-meal diets to improve their acceptability by the fish; egg white was added to the soy diets to increase their protein content. Proximate and elemental analyses were conducted on initial batches of feed from each treatment (Table 2). The moist diets were pelleted by extrusion and then stored at −10°C until needed.

Three soybean-meal diets were supplemented with either EDTA (100 μg EDTA/g: diet 3), zinc sulfate (100 μg Zn/g: diet 5), or EDTA plus zinc sulfate (100 μg EDTA/g plus 100 μg Zn/g: diet 4). The fish-meal and egg-white diets (1 and 7) were both supplemented with zinc sulfate (100 μg Zn/g; Table 1). The eighth diet (7A), which was similar to diet 7 except it contained 440 μg Zn/g, was incorporated in the study for the first 72 d.

TABLE 1.—Diet formulation (% weight composition).

Ingredient	Diet number 1	2	3	4	5	6	7
Fish meal	36.4						
Egg white		13.5	13.5	13.5	13.5	31.3	31.3
Soybean meal		30.3	30.3	30.3	30.3		
Alphacel	8.3					9.7	9.7
Gelatin	4.8					5.0	5.0
Carboxymethyl cellulose	0.40	0.50	0.50	0.50	0.50	0.50	0.50
Wheat starch	1.2	1.3	1.3	1.3	1.3	1.3	1.3
Soy oil	7.1	7.6	7.6	7.6	7.6	7.6	7.6
Tuna viscera		17.7	17.7	17.7	17.7		
Choline chloride, 70%	0.28	0.30	0.30	0.30	0.30	0.30	0.30
Water	34.7	22.1	21.8	21.6	21.8	37.4	37.4
Methionine	0.36	0.38	0.38	0.38	0.38	0.38	0.38
Tryptophan	0.17	0.18	0.18	0.18	0.18	0.18	0.18
Mineral mix[a]	3.8	4.0	4.0	4.0	4.0	4.0	4.0
Zinc solution[b]	0.24			0.25	0.25		0.25
Vitamin mix[c]	1.8	1.9	1.9	1.9	1.9	1.9	1.9
Trace mineral solution[d]	0.38	0.40	0.40	0.40	0.40	0.40	0.40
EDTA[e]			0.25	0.25			

[a] McCollum and Davis salt mixture 185.

[b] Solution contained 39.6 mg Zn/mL ($ZnSO_4$).

[c] Oregon Moist number 2-A, Moore-Clark Company, LaConnor, Washington.

[d] Trace mineral solution contained $Al_2(SO_4)_3 \cdot 18H_2O$, 2.47 g/L; $CuSO_4 \cdot 5H_2O$, 2.04 g/L; $MnCl_2 \cdot 4H_2O$, 7.09 g/L; KI, 1.70 g/L; $CoCl_2 \cdot 6H_2O$, 10.5 g/L.

[e] Contained 39.6 mg EDTA/mL; Na_2EDTA was used to make the solution.

Fish and fish husbandry.—Rainbow trout (mean weight 4.5 g), obtained from the School of Fisheries, University of Washington, Seattle, were placed into 15 700-L fiberglass tanks (125 fish per tank) supplied with 5 L/min of temperature-controlled (13°C) dechlorinated city water. An Oregon Moist Pellet diet was fed for 6 weeks before the experiment to allow for acclimatization. During the study, the experimental diets were fed to duplicate lots of fish.

No attempt was made to equalize the numbers of fish in each tank to account for mortality, which was less than 1% in all tanks; however, all groups were reduced to 75 fish after 4 months to prevent overcrowding. Feeding rates for diet 1 were determined by means of a modified hatchery constant of 40.6 (Buterbaugh and Willoughby 1967), based on growth data from previous experiments with rainbow trout of similar size that were raised under identical conditions and fed a diet equiva-

TABLE 2.—Diet proximate composition and mineral analysis at the beginning of the experiment.

Constituent	Diet number 1[a]	2	3[b]	4[a]	5[ab]	6	7[a]
Proximate composition (%, dry weight)							
Crude protein	54.2	48.6	48.3	48.7	48.8	53.3	53.0
Fat	17.5	13.5	13.7	14.1	14.0	11.8	12.0
Ash	8.4	9.2	9.5	9.3	7.3	7.3	7.2
Mineral composition (μg/g, dry weight)							
Ca	2,370	4,370	4,520	4,550	4,320	3,320	3,160
Co	4	12	11	12	11	10	10
Cu	9	14	12	14	13	3	3
Fe	464	289	295	288	289	265	252
K	4,600	10,100	9,660	10,100	9,230	4,930	5,450
Mg	115	1,480	1,470	1,440	1,410	557	555
Mn	11	21	20	21	19	8	12
Na	9,280	4,870	4,600	4,890	4,480	5,140	5,030
P	14,300	6,840	6,520	6,850	6,280	3,890	3,950
Sr	34	6	5	6	5	2	3
Zn	168	38	41	137	129	4	88

[a] Contained zinc sulfate at 100 μg Zn/g of diet.

[b] Contained EDTA at 100 μg EDTA/g of diet.

lent to diet 1 (Table 1). Feeding rates for the other diets were based on those established for diet 1. Feeding rates were adjusted weekly based on projected growth. Fish were initially fed by hand five times per day; the number of feedings was gradually reduced to twice daily by the time the fish reached 100 g. Throughout the experiment, the fish were fed 7 d of rations in 5 d because no food was fed on weekends. The study was conducted for 202 d. Fish were counted, and the total weight of fish per tank was determined, every 4 weeks.

An additional feeding experiment was conducted to determine zinc levels in blood and whole gills after a single feeding of a high-zinc egg-white diet (2,000 μg Zn/g). Fish weighing 100 g were fasted for 2 d and then fed to satiation in a single feeding period. Ten fish each were sampled at 1, 2, 5, 12, 24, 48, and 72 h postfeeding.

Tissue collection and analysis.—Tissue samples were collected from fish receiving the lowest and highest zinc diets (diets 6 and 7A) on day 72. Analyses of data from this sampling were used to select organs for zinc determination at the end of the experiment on day 202. In all cases, 10 fish from each treatment were randomly selected for tissue analysis.

Prior to sampling, fish were starved for 72 h, then killed by a blow to the head. Tissues used for analysis were removed with stainless-steel instruments and were placed in tared acid-washed glass scintillation vials. Whole blood (0.2–0.5 mL) was obtained by severing the caudal penduncle and allowing the blood to drain directly into an acid-washed scintillation vial. Portions of the following tissues were collected: scales, skin, and muscle from the left side, anterior to dorsal fin; dorsal lobe of the caudal fin; both eyes; left operculum; and complete liver. The remainder of the fish was used for whole-body mineral determination.

After collection, samples were weighed, dried for 24 h in a vacuum oven at 65°C, reweighed, charred under infrared lamps, and then placed in a muffle furnace for 24 h at 550°C. The resulting ash was dissolved in 1 mL HCl and 1 mL HNO_3, diluted to 10 mL with deionized water, and filtered. Elemental analyses were performed with an inductively coupled argon plasma emission spectrometer (Jarrell-Ash Division, Fisher Scientific Company, Waltham, Massachusetts). The concentrations of aluminum, calcium, cobalt, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, strontium, and zinc in fish tissues were determined. All elemental analyses are reported on a dry weight basis.

Analyses of variance were followed by multiple comparisons of tissue elemental values by Tukey's tests (Scheffé 1964) and comparision of two means by Kolmogorov–Smirnov tests (Hollander and Wolfe 1973); regression analyses were performed by the methods described in Snedecor and Cochran (1967). All tests for significance were made at $P < 0.05$ unless otherwise stated.

Results

Selection of Tissues for Determination of Zinc Status

After 72 d, a comparison of tissue zinc concentrations between fish fed the high- and low-zinc egg-white diets (7A and 6) indicated that several tissues were useful for zinc-status measurements based on significant differences between treatments (Table 3). Zinc concentrations in whole blood and gills appear to be correlated with dietary zinc level, but it was found that these levels were transitory. When a high-zinc diet (2,000 μg/g) was given in a single feeding to rainbow trout, zinc levels in blood and gills increased at the first sampling, 1 h after the fish had been fed. Zinc concentrations in gills showed a two- to fourfold increase over the initial concentrations when they peaked between 5 and 12 h after feeding, then slowly declined to baseline levels by 48 to 72 h. As a result of these tests, muscle, whole body, and the caudal fin were the tissues selected for analysis at the conclusion of feeding.

Zinc Concentrations in Tissues (202 d)

Muscle.—Only fish fed the zinc-supplemented egg-white diet (diet 7) had significantly higher muscle zinc concentrations than fish fed diets containing soy or egg without zinc supplementation

TABLE 3.—Zinc concentration in tissues of rainbow trout fed egg-white diets containing 4 or 440 μg Zn/g dry tissue weight (means ± SD for 10 fish). Asterisks denote significantly higher tissue concentrations (P < 0.05*).

Tissue	Diet 6 4 μg/g Zn	Diet 7A 440 μg/g Zn
Blood	63±31	137±17*
Liver	84±13	97±12
Scales	67±33	208±20*
Skin	36±11	96±10*
Eyes	253±81	315±60
Operculum	68±18	133±10*
Caudal fin	88±9.1	186±23*
Gills	84±8.8	545±16*
Muscle	20±5.1	28±4.0
Whole fish	45±10	121±30*

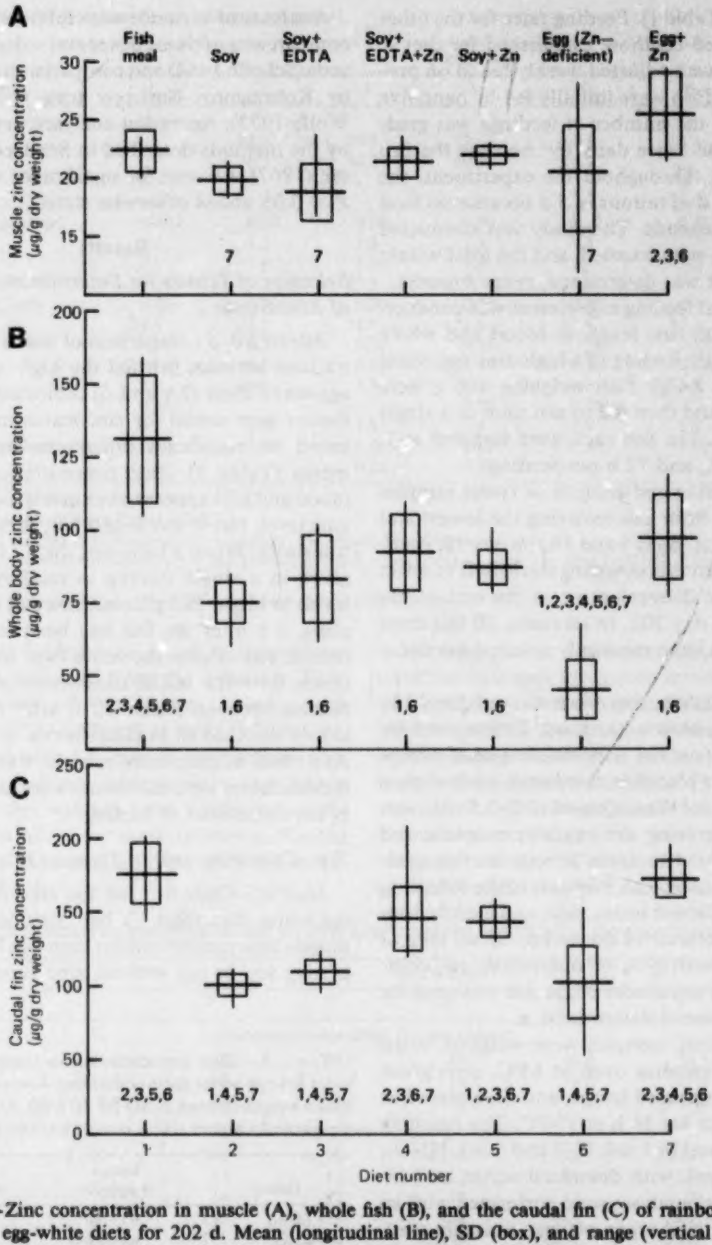

FIGURE 1.—Zinc concentration in muscle (A), whole fish (B), and the caudal fin (C) of rainbow trout fed fish-meal, soy, or egg-white diets for 202 d. Mean (longitudinal line), SD (box), and range (vertical line) are shown. Numbers below each figure indicate the diet numbers that are significantly different at $P = 0.05$.

(Figure 1). However, muscle concentrations were similar in all zinc-supplemented diets. Mean zinc concentrations fell within a small range (18–26 µg/g).

Whole body.—Dietary zinc levels were significantly ($r = 0.86$; $P < 0.05$) correlated with whole-body zinc concentration (Figure 2). Comparisons between groups show that the addition of $ZnSO_4$ to the soybean-meal diet (diet 3 versus diet 4) did not significantly increase whole-body zinc levels

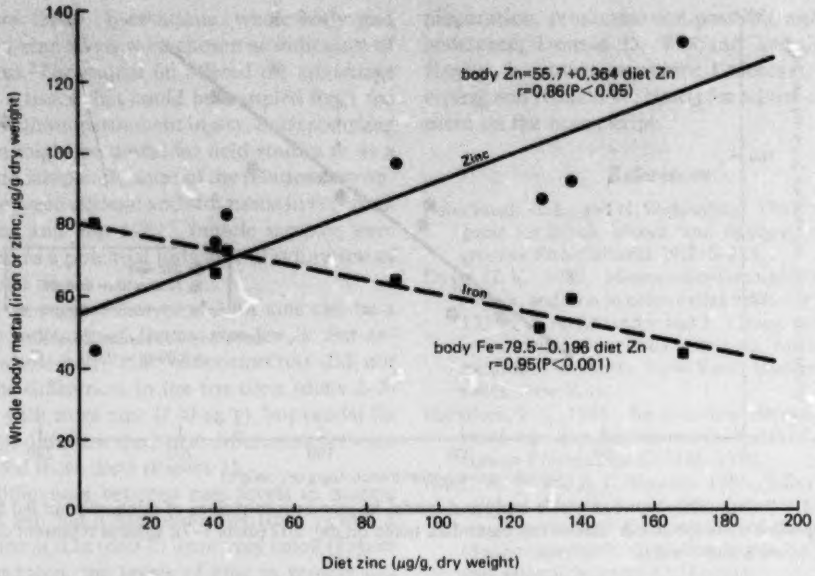

FIGURE 2.—Relationship between whole-body metal concentrations (zinc or iron) and dietary zinc levels.

in the fish fed the soy-based diets (Figure 1). Fish fed diet 1 contained significantly higher zinc concentrations than did fish fed other diets, while fish fed the zinc-deficient egg-white diets had the lowest whole-body zinc values.

Caudal fin.—Like whole-body zinc levels, caudal fin zinc concentrations (Figure 1) reflected dietary zinc levels ($r = 0.84$; $P > 0.05$). Fish fed soybean-meal diets without zinc supplementation contained low levels of caudal fin zinc, while fish fed the zinc-supplemented diet had increased caudal fin zinc. The addition of EDTA to the soybean-meal diets had no significant effect on caudal fin zinc levels. The addition of $ZnSO_4$ to the egg-white diet (diet 7) significantly increased caudal fin zinc concentrations compared to diets 2–6. Zinc levels in fish fed the fish-meal diet (diet 1) were equivalent to zinc levels in fish fed the zinc-supplemented egg-white diet (diet 7). Comparisons of the whole-body and caudal fin zinc values indicated a strong statistical relationship ($r = 0.89$; $P < 0.01$; Figure 3).

Metal Interactions

There was also a significant negative correlation ($r = 0.95$; $P < 0.001$) between whole-body iron and dietary zinc levels (Figure 2), indicating a strong antagonism between these metals. Comparison of mean whole-body zinc concentrations from the seven treatments and whole-body concentrations of the other elements examined indicated that a statistical relationship ($r = 0.87$; $P < 0.05$) exists between whole-body zinc concentrations and whole-body iron concentrations (Figure 4). With the exception of iron and zinc, other whole-body metal concentrations measured did not appear to be statistically interrelated.

Discussion

An ideal organ or tissue for indicating zinc status should respond directly to dietary zinc levels and show a large difference in zinc concentration between the saturated and deficient states. Classically, in mammals, whole body or femur have been the tissues of choice for this purpose. For example, Huber and Gershoff (1970) observed increases in skeletal and soft-tissue zinc levels in rats fed high levels (1,550 μg/g) of dietary zinc (as $ZnCl_2$) in a casein diet; when they fed deficient diets (1 μg/g), reduced zinc levels in the femur were observed. Shah et al. (1971) found that bone zinc levels were elevated when zinc from animal protein or $ZnCO_3$ was fed to rats; however, the soft tissues did not show increased zinc concentration. At present, no indicator organ has been proposed for fish that does not require the destruction of the animal.

While response to dietary zinc or nutritional

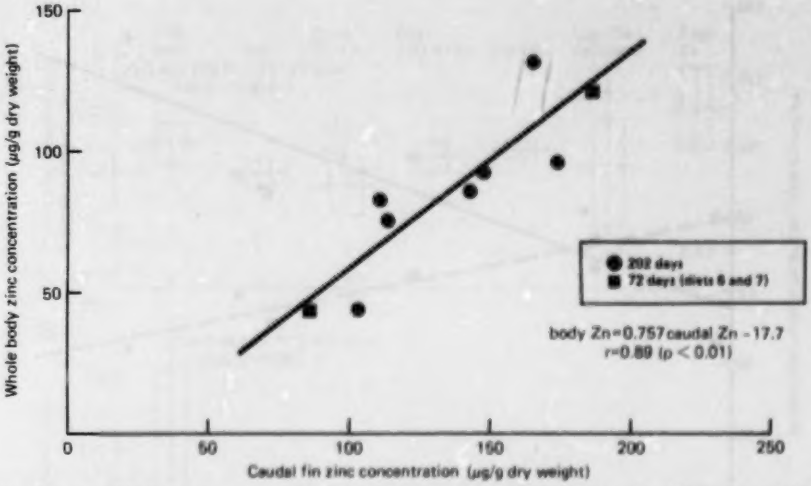

FIGURE 3.—Relationship between whole-body and caudal fin zinc concentrations of rainbow trout fed fish-meal, soy, or egg-white diets for 202 d. Circles represent data taken on day 202 (diets 1–7); squares represent data taken on day 72 (diets 6 and 7 only).

zinc status is the key function of an indicator tissue or organ, it is also useful to know both maximum (saturation) and minimum (deficient) levels of zinc in the chosen tissue. In most circumstances, diagnostic methods for confirming zinc deficiency are more important than determining saturation of the metal in the animal. Nevertheless, knowledge of the saturation of a specific tissue with zinc can be useful in determining zinc status. For example, Jeng and Sun (1981) found that, when common carp (*Cyprinus carpio*) were fed high levels of $ZnSO_4$, zinc levels increased in skeletal tissue before it did in muscle tissue. In their study, muscle levels did not increase until the skeletal tissues were saturated, which required 8 weeks of feeding a diet containing 1,007 μg Zn/g.

In our study after 72 d, examination of body organs and tissues (Table 3) of fish fed a zinc-deficient diet (4 μg/g) or a high-zinc diet (440 μg/g) indicated that certain organs were more sensitive than others to the dietary regime and, therefore, they might be useful in determining zinc sta-

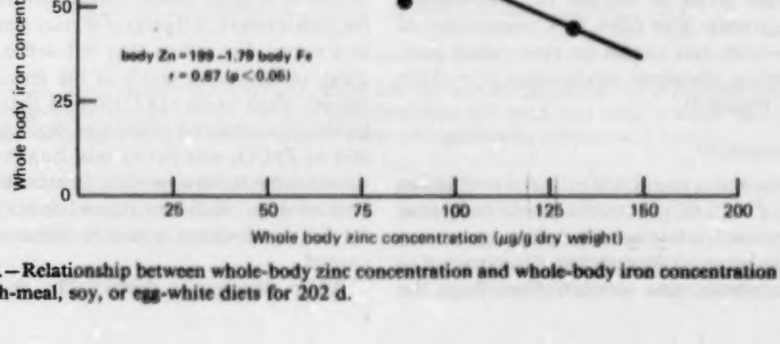

FIGURE 4.—Relationship between whole-body zinc concentration and whole-body iron concentration of rainbow trout fed fish-meal, soy, or egg-white diets for 202 d.

tus. From those observations, whole-body and caudal fin zinc levels were chosen as indicators of zinc status. The caudal fin offered the advantage of being a tissue that could be sampled from the live fish without permanent injury. Such sampling of the fin might be useful for field studies or as a diagnostic sample. Because of the relationship observed between skeletal and soft tissue in common carp (Jeng and Sun 1981), muscle samples were also taken as a potential indicator of saturation of the skeletal tissue.

Our data suggest that caudal fin zinc can be a sensitive indicator of dietary zinc levels. For example, whole-body zinc concentrations did not reflect the differences in the soy diets (diets 2–5) fortified with extra zinc (100 μg/g), but caudal fin zinc levels did show statistical differences between the fish fed these diets (Figure 1).

The differences between zinc levels in muscle tissue of fish fed a high-zinc diet (diet 7) and a zinc-deficient diet (diet 6) were very small (Figure 1). In addition, the levels of zinc in muscle did not change significantly between day 72 (Table 3) and day 202 (Figure 1). Therefore, it appears that approximately 20 μg Zn/g is the minimum zinc concentration of functioning muscle tissue in rainbow trout. In a similar way, minimum zinc levels in the caudal fin may be near 86 μg/g (Figure 1). On the other hand, whether or not the hard tissues, such as caudal fin, were saturated with zinc can only be surmised because it was not directly addressed in the experimental design. Based on data collected from fish fed the high-zinc diet (diet 7A, 440 μg/g) for 72 d (Table 3), it would appear that the caudal fin becomes saturated at approximately 200 μg/g.

It has been reported that zinc can antagonize iron in mammals (Smith and Larson 1946; Settlemire and Matrone 1967a, 1967b; Davis 1980). Ogino and Yang (1978) found whole-body iron levels increased in rainbow trout that had low whole-body zinc. The strong inverse correlation between dietary zinc and whole-body iron (Figure 2) and whole-body zinc and whole-body iron (Figure 4) found in our study reflects a similar mechanism in rainbow trout. Since we have reported that fish diets can be supplemented with zinc at very high levels (1,000–2,000 μg Zn/g; Wekell et al. 1983), the impact of the use of these high levels of zinc on other metals in fish must be considered.

Acknowledgments

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Survival of *Edwardsiella ictaluri* in Pond Water and Bottom Mud[1]

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Abstract.—*Edwardsiella ictaluri,* the causative agent of enteric septicemia of catfish, is involved in about one-third of all disease cases in diagnostic laboratories where channel catfish (*Ictalurus punctatus*) is the predominant species examined. *Edwardsiella ictaluri* causes disease primarily when water temperatures range from 18°C to 28°C; these temperatures prevail during May and June and again in September and October in southern USA where catfish are cultured. This investigation was undertaken to confirm earlier reports that *E. ictaluri* does not survive in water and to determine if it could survive in bottom muds of ponds. Survival of *E. ictaluri* was determined in pond water at 5°C and 25°C, and in bottom mud at 5°C, 18°C, and 25°C. In mud incubated at 25°C, *E. ictaluri* maintained itself at densities of $10^{6.5}$ cells/mL for 95 d; at 18°C the organism maintained approximately 10-fold higher densities for 40 d. However, *E. ictaluri* survived for less than 10 d in water at 25°C and for less than 15 d in water and mud at 5°C. These data demonstrate that *E. ictaluri* can survive in pond bottom muds for an extended period of time; from this refuge, it may be a source of infection from spring through the fall. In view of these data, it is unlikely that *E. ictaluri* is a true obligate pathogen, but its survival is restricted by environmental conditions.

Enteric septicemia of catfish (ESC) is caused by the enteric bacterium *Edwardsiella ictaluri,* and it is one of the most serious infectious diseases of pond-cultured channel catfish (*Ictalurus punctatus*). The disease occurs primarily in the spring and early summer and again in the fall when water temperatures are between 22°C and 28°C (Plumb 1984). Hawke (1979) suggested that *E. ictaluri* is an obligate pathogen because it did not survive very long in water. Plumb and Sanchez (1983) further proposed that *E. ictaluri* is an obligate and host-specific pathogen due to its ability to infect only channel catfish. This study further investigated the ability of *E. ictaluri* to survive in pond water or bottom mud at 5°C, 18°C, and 25°C.

Methods

Pond mud and water were taken from a production pond containing channel catfish. Mud and water (250 mL of each) were placed into 500-mL flasks and sterilized at 121°C under pressure of 1.41 kg/cm^2 for 15 min. After cooling, each substrate was inoculated and thoroughly mixed with 5 mL (10^8 cells/mL) of an 18-h brain–heart infusion (BHI) broth culture of *E. ictaluri.* One flask each of mud and water were placed at 5°C and 25°C. The *E. ictaluri* was from a clinical case of enteric septicemia of catfish (Southeastern Cooperative Fish Disease Project Case GA 80-54), which had been lyophilized and reconstituted on BHI agar 24 h before use.

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Each inoculated sample was assayed for *E. ictaluri* at the time of inoculation (day 0), on days 1, 3, 5, 7, 10 after inoculation, then at 5-d intervals through day 45, and finally at 10-d intervals through day 95. One milliliter of water or mud was aseptically removed from each flask and diluted in serial 10-fold dilutions to 1:100,000 in sterile water. *Edwardsiella ictaluri* was enumerated by making five separate 10-μL drops of each dilution on a BHI agar plate. Dilutions from which three to 30 colonies developed per drop after 48 h incubation at 25°C were used to calculate the number of bacteria per milliliter of water or mud. Periodically, cultures isolated from mud at 25°C were checked for biophysical and biochemical conformity to *E. ictaluri* (Hawke et al. 1981). Each temperature regime of each substrate was terminated when no *E. ictaluri* were isolated for two consecutive sampling periods or when a sample became contaminated with another bacterium; however, the 25°C mud treatment was terminated at day 95.

In a second study, mud was inoculated with *E. ictaluri* as previously described and incubated at 18°C and 25°C. Bacterial counts were made at 0, 1, 5, 10, 20, 30, and 40 d postinoculation.

Results and Discussion

At day 0, the numbers of *E. ictaluri* were $10^{6.44}$ cells/mL of mud and $10^{6.29}$ cells/mL of water. *Edwardsiella ictaluri* numbers in 5°C water remained at the same level as the inoculum at day 1 and then began to decrease until no bacteria were isolated on day 15 (Figure 1). In 25°C water, *E. ictaluri* numbers increased to $10^{6.44}$/mL at day 1 but then began to decrease, and at day 10 no bacteria were isolated.

Edwardsiella ictaluri in mud incubated at 5°C responded as it did in 5°C water (Figure 1). The number of bacteria increased at day 1 then decreased rapidly to zero at day 15. However, numbers of *E. ictaluri* in the 25°C mud increased at day 1 and peaked at day 3 ($10^{7.67}$ cells/mL) before declining to an average $10^{6.33}$ cells/mL through day

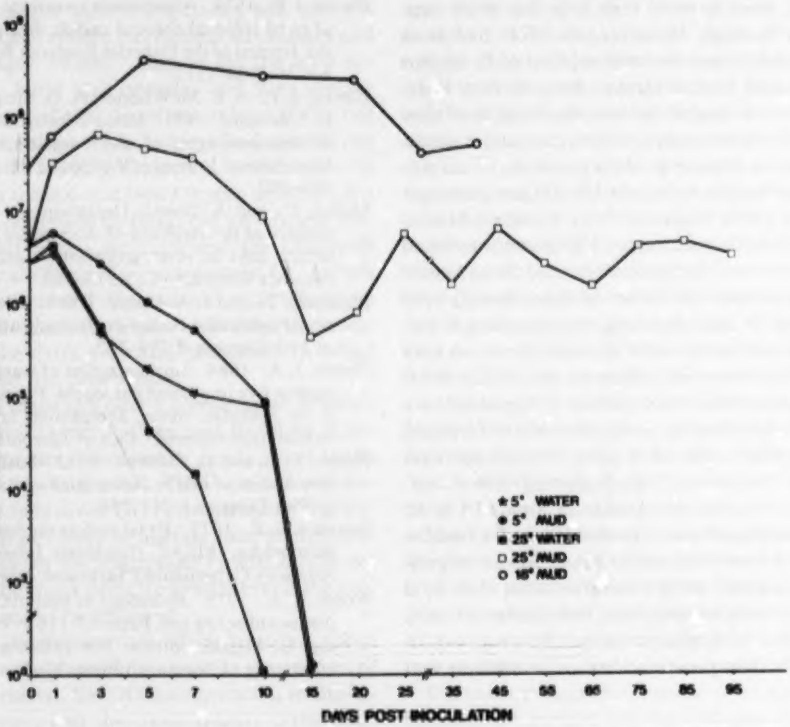

FIGURE 1.—Survival of *Edwardsiella ictaluri* in pond water and mud when incubated at 5°C, 18°C, or 25°C.

95. At that time the treatment was terminated because no appreciable change in cell counts had occurred between days 25 and 95.

In the second set of experiments, mud was incubated at 18°C and 25°C with an initial inoculum of $10^{7.2}$ cells/mL. The number of *E. ictaluri* in the 18°C mud rose to $10^{8.3}$ by day 5 and dropped to $10^{7.3}$ on day 30 (Figure 1). This regime was terminated at day 40 because of contamination. Numbers of *E. ictaluri* in the 25°C mud remained very close to those of the first experiment; therefore, it was terminated at day 50.

Edwardsiella ictaluri forms a very distinct conical-shaped colony with irregular flat sides when viewed under a dissecting microscope after 24 and 48 h incubation. The colony is white and slightly opaque. These characteristics allowed rapid detection of contaminating bacteria. Colonies in the 25°C mud were confirmed as *E. ictaluri* to insure the accurate recognition of the typical colonies during the lengthy survival period.

It was suggested by Hawke (1979) that *E. ictaluri* is probably an obligate pathogen. He had performed limited experiments to determine the survival of *E. ictaluri* in water and reported that it did not survive more than 8 d. Our study supports his findings. However, no effort had been made to determine the survivability of *E. ictaluri* in pond mud. Our evidence indicates that *E. ictaluri* survives in mud for extended lengths of time at 25°C. For many years, *Aeromonas salmonicida,* the causative organism of furunculosis of salmonids, was thought to be a strict obligate pathogen (Snieszko 1973; Wolke 1975). However, Michel and Dubois-Darnaudpeys (1980) demonstrated that *A. salmonicida* survives in mud for at least 9 months and does not loose virulence during mud incubation. It had also been thought that *A. salmonicida* did not survive in water for more than a few days. However, Allen-Austin et al. (1984) demonstrated that this organism dropped below a platable count level in water after about 16 d, but could be recovered 14 d later if nutrients were added to the water. They suggested that *A. salmonicida* becomes dormant after about 14 to 16 d if no fish are present, but that it is still available to infect fish several weeks later. In our experiment, *E. ictaluri* did not survive more than 15 d in water at any temperature, but rejuvenation by the addition of nutrients was not investigated. In view of the data presented here, it is unlikely that *E. ictaluri* is a strict obligate pathogen, but its survival outside of the host is probably restricted to certain environmental conditions.

Miyazaki and Plumb (1985) proposed that channel catfish become infected with *E. ictaluri* through their nasal openings. The reservoir of *E. ictaluri* in the mud could be a source of this pathogen, which would partially explain the fall outbreaks of enteric septicemia in channel catfish after the disease had been controlled by chemotherapy during earlier epizootics. The ability of *E. ictaluri* to survive for long periods of time in bottom muds presents a problem for controlling the disease. It suggests that disinfection of pond bottoms should be done between crops of fish. However, this is not possible where partial harvesting and replacement stocking with fingerlings is practiced. In such instances, medicated feed should be applied at the first sign of *E. ictaluri* infection.

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Design and Application of Vacuum Degassers

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Abstract.—Designs of vacuum degassers, which alleviate gas supersaturation in hatchery waters, have progressed greatly in recent years. Basic rules of thumb now exist by which the feasibility of vacuum degassers can be determined for particular facilities, and by which complete systems can be designed and built to meet local needs. These rules are presented in the form of step-by-step procedures and guidelines directed to practicing hatchery operators. The flexibility of vacuum degasser designs is illustrated for large-, medium-, and small-volume systems.

Three of the more popular methods currently used for degassing supersaturated water are packed columns, oxygen injection systems, and vacuum degassers. Each system has distinct advantages and disadvantages. Packed columns are the least expensive to build and operate, and they replace some of the depleted dissolved oxygen (DO), but the lowest total gas level that a packed column can provide is 101–102% saturation, which may be too high in some situations. Oxygen injection is a promising degassing method, but an efficient system has not been developed and the long-term physiological effects of supersaturated DO on fish must be determined. Operational costs of oxygen injection are generally higher than other methods, although the extra cost may ultimately be offset by increased fish production.

Vacuum degassers offer a compromise between the other two systems. They cost less than injection systems, and consistently produce gas levels that are below saturation. Their main disadvantage is that they lower DO concentrations slightly. They are more costly to operate than packed columns, although the magnitude of added cost depends on the water pressure available and the volume of water to be degassed.

Among the factors that affect decisions about which degassing method to use are the species of fish being reared, the DO concentration in the incoming water, and the water quality criteria required at the facility. If 102% total gas pressure is acceptable, packed columns are recommended. If gases must be below saturation and the DO concentration in the incoming water is acceptable, vacuum degassing may be used. If incoming DO level is low, either an oxygen injection system or a combination of packed columns and vacuum degassers in series can be used.

The art of vacuum degasser design has evolved to the point that nearly any fish culturist can build a successful unit. In this paper, I offer general non-engineering rules of thumb that can be used to design and build a working vacuum degasser.

Basics of Vacuum Degasser Design

If water is supersaturated, a pressure difference exists between the atmosphere and the dissolved gases. Given enough time, the gases will come into equilibrium without the aid of degassing equipment. Packed columns accelerate the degassing process by exposing thin films of water to the atmosphere. Unfortunately, the closer to equilibrium the dissolved gas levels become, the more the process slows, and typical packed columns only approach 101% saturation, at best. A vacuum degasser is simply a packed column with sealed ends (Figure 1). A vacuum pump is used to reduce the internal pressure, allowing more gas to be removed as it approaches the lower equilibrium level.

When designing vacuum degassers, one must observe two critical factors: the flow rate through the columns, and the vacuum level. Flow rates of existing degassing units range from 50 to 200 gal/min per square foot of cross-sectional area of the column. The most successful vacuum degassers are designed for a flow rate of 100 gal/min·ft^2. Higher flow rates require higher vacuums, resulting in increased operational costs. Values for vacuum levels typically range from 3 to 8 in of mercury; 5 in Hg is the most common.

Most vacuum degassers are designed and constructed to operate at a constant flow and vacuum level. It is possible to alter these variables slightly (e.g., to reduce flow rate) but it can be a delicate maneuver to balance acceptable gas levels with the new incoming flow rate and vacuum level.

If a vacuum degasser is made by simply sealing the ends of a packed column and attaching a vacuum pump, water does not flow out the bottom

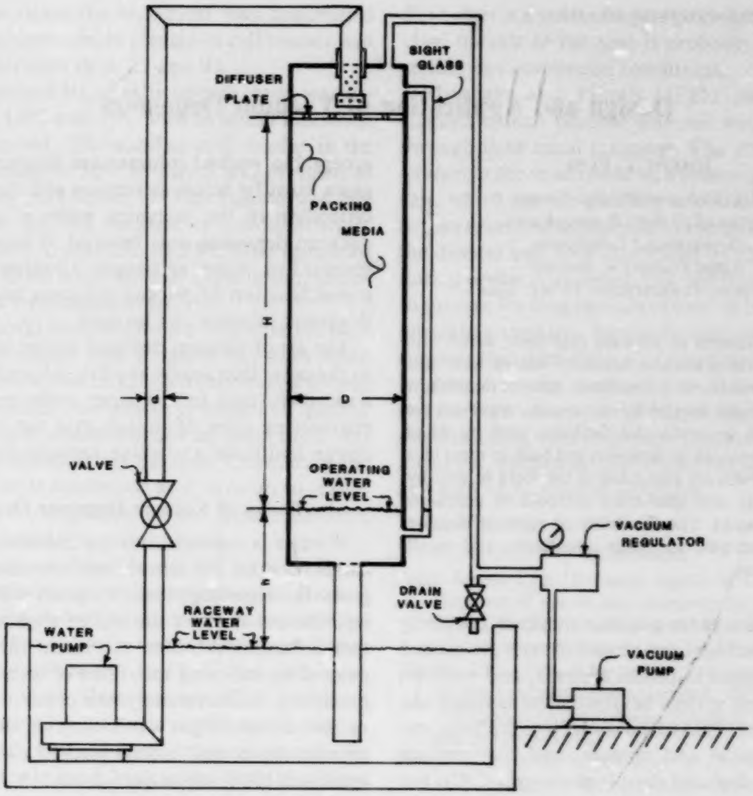

FIGURE 1.—Typical vacuum degasser design; diameter of outlet pipe is approximately 1.4 × d.

because of the negative pressure developed in the unit. A pump could be used to extract the water, but gravity is a more dependable and less complicated choice. A pipe is attached to the bottom of the degasser, and the weight of the water falling through the pipe is enough to counteract the vacuum, provided the pipe is long enough. To determine the pipe's length (h, Figure 1), one can apply the general rule that 1.1 ft of water is needed for each inch (Hg) of vacuum. For a degasser operated at 5 in Hg vacuum, then, 5.5 ft of water are required at the bottom to enable gravity to overcome the vacuum. If the operating vacuum is increased, the water column must be lengthened accordingly. Note that the dimension h is measured from the operating water level in the degasser to the operating water level at the discharge—be it a raceway, sump, or other structure.

This height requirement can result in higher operational costs for vacuum degassers than for packed columns. Facilities that do not have sufficient water pressure (about 5 lb/in^2) must pump the water to the top of the degasser, which is expensive. The approximate cost of operating a pump (1985 prices) was about $1 per day per horsepower (hp). Large vacuum degassers generally require 5-hp pumps for 1,000-gal/min units. The operation of a 5-hp pump costs about $150/month. If water pressure in the supply line is adequate, of course, pumping is unnecessary and operational costs of the degasser are smaller.

As in packed columns, bulk media are used in vacuum degassers to increase the exposure of water to the atmosphere within the chamber. The bulk medium that appears most economical and effective consists of plastic rings 1.5 inches in diameter—although rings 2 inches in diameter are acceptable when debris is a problem. Usually an exposed height (H in Figure 1) of 4–5 ft is used. A perforated plate or pipe diffuser above the medium spreads the water evenly. A rather elaborate diffuser plate for a large application is shown in

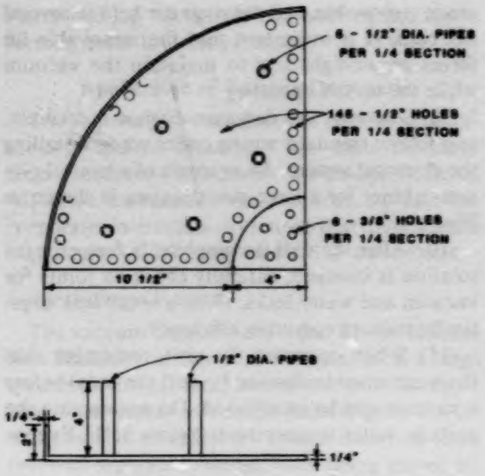
5 - 1/2" DIA. PIPES PER 1/4 SECTION
146 - 1/2" HOLES PER 1/4 SECTION
6 - 3/8" HOLES PER 1/4 SECTION
10 1/2"
4"
1/2" DIA. PIPES
1/4"
1/4"

FIGURE 2.—Diffuser plate designed for a large vacuum degasser (¼ section).

Figure 2. In the absence of a diffuser, the water channels down through the unit, making most of the medium ineffective. Depending on the water quality, it may be advisable to make the medium easily accessible for cleaning.

In recent degasser designs, the vacuum is maintained by very small, continuously running vacuum pumps that operate in conjunction with a vacuum regulator. Columns sized for 1,000 gal/min water flow use $\frac{1}{15}$-hp pumps. In earlier units, several large pumps, arranged in tandem, and vacuum storage tanks were used, allowing the pumps to cycle. The intention was to extend the life of the pumps and also to serve as a safety measure if a pump failed. However, the additional equipment (tanks, regulators, and switches) is unnecessary. If a vacuum pump fails, the degassers continue to perform as packed columns, discharging water at about 102% total gas pressure. The extracted gas is discharged from the column in the form of large bubbles, along with the water.

Another possible point of failure is the water supply pump. It is prudent to design a system that automatically maintains water flow to the rearing units if a failure occurs. Several designs accomplish this objective. The most common is to locate the pump intake at the head end of the rearing unit. The discharge from the degasser is returned to the rearing unit below the pump intake and blended with the untreated water. If a unit fails, all water simply flows past the degasser.

If partial degassing of the water is not sufficient, a similar system can be used that incorporates a weir. The weir prevents untreated water from bypassing the unit unless the pump fails. This system degasses nearly all the water flow to a rearing unit.

Design Considerations for Vacuum Degassers

I suggest taking the following steps to determine if a vacuum degasser is needed, if it would be economically feasible, and, finally, what its basic design characteristics should be for a particular facility.

(1) Determine whether it is necessary to keep total dissolved gas levels at or below saturation. If 101–102% total gas saturation is acceptable, packed columns should be used.

(2) Determine whether incoming DO levels are high enough to allow a decrease of up to 0.5 ppm without a major impact on the fish. If they are, a vacuum degasser can be used. If they are not, an oxygen injection system or combination of packed column and vacuum degasser should be considered.

(3) If a decision is made to build a vacuum degasser, determine the volume of water, in gallons per minute (GPM) that must be degassed. Calculate the approximate column diameter (D, Figure 1), in inches, by the equation: $D = 1.35 \times \sqrt{GPM}$. This gives a minimum column diameter that allows a flow of 100 gal/min·ft^2. For practicality, choose a column diameter of the next larger commonly manufactured dimension. Chambers with a 12-in or smaller diameter are generally made of schedule-40 steel or plastic pipe. Larger columns are also made of steel pipe, but the wall thickness must be at least 0.375 in to prevent the internal collapse of the structure if a full vacuum develops.

(4) Determine the incoming water pressure to be used for the vacuum degasser. If it is at least 5 lb/in^2, a pump is not required.

(5) If a pump is required, determine the approximate horsepower and monthly operating expense (Table 1). If this expense is affordable, select a pump that is at least 65% efficient. An industrial pump representative can help select an appropriate pump if given details about flow rate, pressure required (12 ft of head), the electrical service available, and such other features as can be shown in a sketch of the system. If the use of brass or bronze in the water supply is prohibited, specify an all-iron pump. Pump motors of 3 hp or less generally use single-phase, 115-V electrical service. Three-phase electrical service is desirable for larger mo-

TABLE 1.—Pipe sizes, approximate pump sizes, and pump operating costs for vacuum degasser designs.

Flow rate (gal/min)	Pipe diameter[a] (in)	Approximate pump size (horse-power)	Estimated monthly operating cost (1985 dollars)
0–50	2	¾	23
50–150	3	¾–2	23–60
150–400	4	2–3	60–90
400–800	6	3–5	90–150
800–2,000	8	5–15	150–450

[a] Measurement d in Figure 1.

tors; if three-phase service is unavailable, phase converters may be installed if the cost is justified.

(6) Size water lines (d, Figure 1) correctly (Table 1). The water lines can be made of either steel or plastic pipe. The vacuum piping should be at least ½ inch in diameter; it should be insulated and heat-taped to prevent winter freeze-up, and it should be sloped to allow moisture to drain. A typical design for vacuum piping is shown in Figure 3.

(7) Obtain a vacuum pump and regulator for the system. A $\frac{1}{15}$-hp pump is sufficient, but larger pumps can be used. Rotary vane-type pumps are ideally suited for this application. The regulator must be able to maintain the desired vacuum within ±½ in Hg. A source for the regulator is Fisher Controls, Marshalltown, Iowa (Model Y612-1: 0–5.5 in Hg, $\frac{7}{16}$-in port, ¾-in pipe thread).

(8) The column packing should be 1½-in-diameter plastic rings (i.e., Koch® or Pall® rings). If the water supply contains suspended matter, a method of removing the rings for cleaning should be provided. Generally, the top of the column is made removable, and the rings are held in several net bags. It is important that the removable lid forms an air-tight seal to maintain the vacuum while the unit is operating.

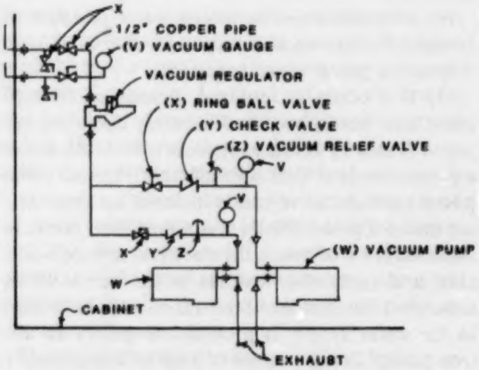

FIGURE 3.—Typical vacuum piping installation for a vacuum degasser system with two columns.

(9) Construct the degasser, anchor it securely, and follow standard wiring codes when installing the electrical system. An example of a control system cabinet for a large-size degasser is shown in Figure 4.

(10) After the unit is complete, before pipe insulation is installed, carefully check all joints for vacuum and water leaks. Even a small leak drastically reduces operating efficiency.

(11) When operating the unit, remember that the water must be flowing (to seal the ends) before a vacuum can be established. Do not operate the units at water temperatures below 35°F. Evapo-

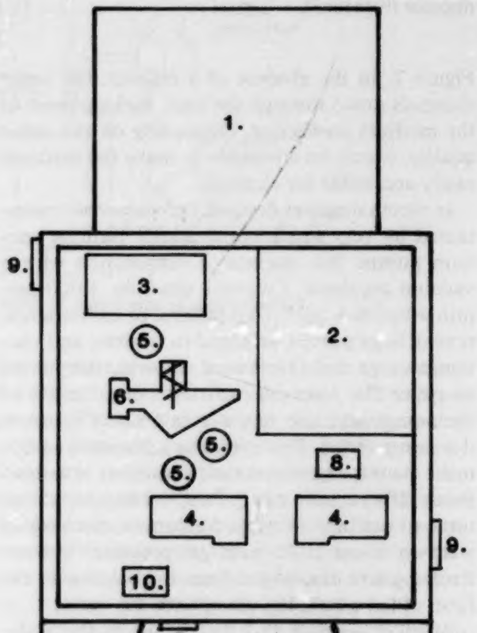

FIGURE 4.—Typical control cabinet arrangement for a large vacuum degasser system: (1) phase converter (if used), (2) circuit breaker panel, (3) motor starter, (4) vacuum pump, (5) vacuum gauge, (6) vacuum regulator, (7) thermostat, (8) heat cable switches, (9) louver and filter, and (10) 120-V power receptacle.

rative cooling within the chamber causes ice to form at these low temperatures.

Application of Vacuum Degassers

The following are examples of current state-of-the-art vacuum degassers. The units represent large-, medium-, and small-volume applications. All follow standard design practices, but each incorporates interesting deviations that illustrate the high degree of flexibility allowed in the criteria.

Large-Volume System

The vacuum degassers at Jordan River National Fish Hatchery in Elmira, Michigan, form a large system (3,400 gal/min) meant to treat a facility's total water supply at a single point (Figure 5). Actually most of this hatchery's water is derived from two well-separated springs, each being served by an independent degassing system. To degas each spring, a portion of the flow is extracted and passed through twin vacuum columns. The columns are 42 inches in diameter and are supplied with about 850 gal/min by 3-hp water pumps. The discharge is mixed with the remaining spring water flow to supply the hatchery. Average dissolved nitrogen decreased from 104% to 92% and DO decreased from 9.4 ppm to 9.0 ppm during initial tests. Water temperature was about 47°F.

Several design features of the Jordan River degassers are notable. A useful item is the window-type sight glass located along the column. An occasional glance at the window shows whether proper flow and water level are being maintained and whether the medium requires cleaning. External (tubular) type sight glasses are not recommended in areas where freezing is possible. The water supply line for each unit is located in the center of the column, keeping the top of the chamber free of piping. This arrangement allows easy access to the medium through the top.

In addition to the $\frac{1}{15}$-hp pumps, the vacuum system incorporates a single low-pressure high-volume air blower. The suction side of the blower is valved to each chamber and is used for rapid evacuation of the cylinder during start-up. This saves time and reduces wear on the smaller vacuum pumps.

A single vacuum regulator is used to serve both chambers, reducing the cost of construction. Valves allow either chamber to operate individually while the other is being serviced.

Medium-Volume System

Rather than using a single large vacuum degasser to treat a facility's entire water supply, another approach often taken is that of degassing water for individual raceways. Small columns are located at the head of each raceway, degassing a portion of the flow and blending it with tne untreated water. This was the approach used at Hiawatha National Fish Hatchery in Brimley, Michigan (Figure 6).

The Hiawatha units, with a flow of 500 gal/min,

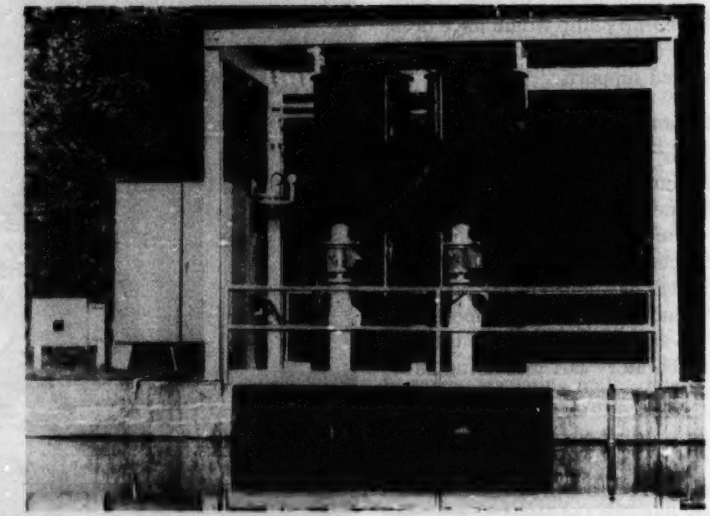

FIGURE 5.—Vacuum degasser installation at Jordan River National Fish Hatchery, Elmira, Michigan.

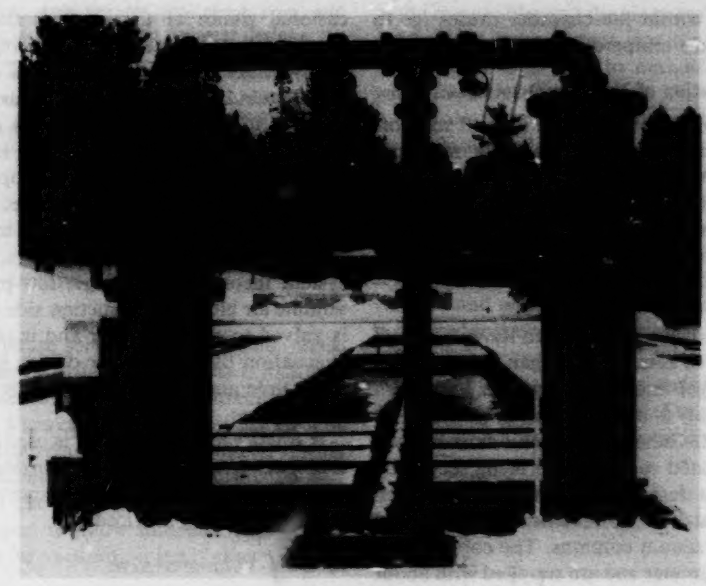

FIGURE 6.—Vacuum degasser installation at Hiawatha National Fish Hatchery, Brimley, Michigan.

are considered typical of intermediate-sized vacuum degassers. The 30-in-diameter columns have a cross-sectional flow of 101 gal/min·ft^2 and operate at a vacuum of 5 in Hg. Initial testing of the system showed a reduction of dissolved nitrogen from 104% to 93% and a reduction of DO from 10.2 ppm to 9.4 ppm at about 40°F. A somewhat unusual feature is the use of a single 5-hp water pump to supply two columns side by side. If supplied individually, a 3-hp pump would have been required for each column, as well as more piping, valves, and associated supports.

Small-Volume System

At many locations, supersaturation occurs only periodically, and managers may choose to degas only such critical water supplies as those supplying rearing units for swim-up fry. For this application, a small portable vacuum degasser capable of handling 100 gal/min was developed at the National Fishery Research and Development Laboratory, Wellsboro, Pennsylvania (Figure 7). The entire unit is mounted on a laboratory cart measuring 28 × 42 in, and can be easily moved. Water pumps (¾

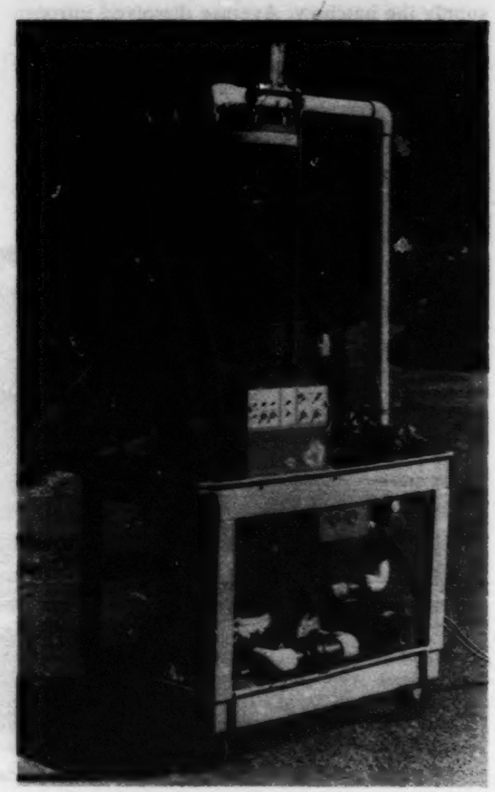

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FIGURE 7.—Portable vacuum degasser developed at the National Fishery Research and Development Laboratory, Wellsboro, Pennsylvania.

hp) are provided for both supply and discharge lines, making the unit independent of local water pressures. The column is 12 inches in diameter and is constructed of schedule-80 polyvinyl chloride pipe. The supply and discharge lines are 2 inches in diameter.

An important feature of this degasser is the control system (Figure 7). The unit can be operated manually by using the sight glass in association with motorized water and vacuum valves, or the operator may choose an automatic mode. At the flip of a switch, a preprogrammed computer monitors water levels, controls pumps and valves, and monitors discharge gas concentrations. The automatic system also provides alarms for high water levels in the column and high dissolved gas in the discharge water.

Acknowledgments

I thank the engineers of the U.S. Fish and Wildlife Service for their work in developing the design criteria for vacuum degassers. In particular, Bill Miller and Jim Kelley of the Region 3 Fort Snelling Office, and Terry McLaughlin of the Denver Engineering Center have been very generous in sharing their experiences.

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Practical Field Methods of Sexing Largemouth Bass

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Abstract.—Several rapid and simple methods of sexing live mature largemouth bass (*Micropterus salmoides*) were assessed: the presence or absence of a swollen reddish genital papilla, the shape of the scaleless area surrounding the urogenital opening, and the depth and angle of probe penetration into the urogenital opening. The genital papilla method was 89% successful during the spring, but only 48% successful during the fall. The shape of the urogenital opening was a poor indicator of sex (53% of the fish were sexed correctly). Probing the urogenital opening was the best single method; it provided success rates of 90% (probe depth) and 94% (probe angle). The highest rate of sexing success (98%) was realized during the spring spawning season from a combination of the papilla and probing methods. The probing methods that were used successfully in this study on largemouth bass might be used with similar success in sexing other fish species.

It is often desirable for biologists and fish culturists to be able to sex fishes without sacrificing them. Sexing any individual fish is a binomial concept and, hence, without a graded scale of success. In sexing numbers of fishes, however, success may be graded. In population studies, it is often only necessary for a sexing method to approach some level of success to be considered applicable. In precise breeding programs that require no error in determining sex, however, procedures used to identify each sex must be applicable to the stringency of the binomial concept.

Different methods of sexing live largemouth bass (*Micropterus salmoides*) have produced varying levels of success. Using hematocrit values to separate the sexes, Steucke and Atherton (1965) found that 52% of the largemouth bass in their sample had to be discarded to ensure the method's overall accuracy. Snow (1963) noted that by a combination of stripping eggs or milt, estimating fullness of the abdomen, and noting the presence or absence of a urogenital papilla (i.e., swollen vent), one could separate the sexes at spawning time. Toots (1972) also recommended using the presence or absence of a urogenital papilla to sex largemouth bass at spawning time and, additionally, noted that body coloration was sometimes helpful in sexing spawning males. Shape of the scaleless area surrounding the urogenital opening was recommended by Parker (1971), and later was criticized by Manns and Whiteside (1980), as a means of sexing largemouth bass. Glass et al. (1962) used a urogenital-probing technique to accurately sex (100% correct) 11 largemouth bass. Driscoll (1969) used an otoscope to successfully sex 97% of a large sample ($N = 154$) of pre- and postspawning bass. The otoscope served as both a probe and viewing aid and, although the latter role was not discussed, probing the urogenital opening was described as being important.

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During stock assessment of largemouth bass populations in selected lakes throughout Connecticut, we found it necessary to determine the sex of fish. Because killing many of these valuable bass was undesirable, we needed a rapid and reliable method of sexing live fish in the field. Therefore, we compared several practical sexing methods. Because the primary goal of this effort was to acquire a quick, easy, and relatively accurate (≈90%) method of sexing live fish for our assessment purposes, an exhaustive evaluation of all potential methods was not undertaken.

Methods

Four solitary methods of sexing largemouth bass were assessed: (1) noting the presence (on females) or absence (from males) of a genital papilla, a reddish protuberance surrounding the urogenital opening; (2) examining the shape of the scaleless area surrounding the urogenital opening (round in males, oval in females) when the fish is held upside down (Parker 1971); and noting (3) the depth, and (4) the angle of penetration of a 1-mm-diameter plastic broom straw inserted into the genital duct (Glass et al. 1962). In females, the probe passes through the oviduct and deep into the ovary at an angle oblique to the main body axis; in males the probe generally does not enter the genital duct, but rather passes a short distance perpendicular to the main body axis into the urinary bladder (Figure 1).

We also evaluated these methods in combinations of, first, the two probing techniques (3 and 4), and, second, the gential papilla and the two probing techniques (1, 3, and 4). In order for sex identification to be made by the combined techniques, each technique had to predict the same outcome. If predictions were incongruent, one observation had to be considered inconclusive and the sex was predicted by the remaining explicit observation.

Largemouth bass were collected by electrofishing between the spring of 1981 and the fall of 1982 in three Connecticut lakes: Candlewood Lake, Lake Lillinonah, and Pachaug Pond. After sex was predicted by the above methods, it was verified by dissection and examination of the gonads; fish deemed immature (based on shape and size of gonads) were omitted from the results (the smallest male and female fish included were 272 mm and 300 mm, respectively).

Results and Discussion

The success of the genital papilla method as a means of identifying sex varied seasonally. During the spring spawning period, most mature females developed a highly noticeable eurythemic papilla and the method proved almost 90% effective (Table 1). During the fall, however, the papilla apparently atrophies and, correspondingly, the method greatly overestimated the number of males. In contrast, McComish (1968) was able to sex mature bluegills (*Lepomis macrochirus*) throughout the year with absolute success even though seasonal variation in papilla size was noted.

The shape of the scaleless area surrounding the urogenital opening was a poor indicator of sex, and was not much better than chance (53% correct) in predicting sex correctly (Table 1). These results corroborate those of Manns and Whiteside (1980) who judged this method to be of limited value in sexing Guadalupe bass (*Micropterus treculi*) and largemouth bass (67% accurate for fish 355 mm or longer). Both the present study (Table 1) and that of Manns and Whiteside (1980) indicate that this method grossly overrepresents males. Al-

TABLE 1.—Percent of largemouth bass correctly sexed by six different methods. Sample sizes are in parentheses.

Method (season)	Male	Female	Sexes combined
A: Presence or absence of red papilla (1981–1982)[a]			
May–Jun	97 (98)	79 (79)	89 (177)
Sep–Nov	95 (65)	48 (67)	71 (132)
B: Shape of urogenital region (spring and fall 1981)[b]			
Fish 300–355 mm	51 (37)	46 (24)	49 (61)
Fish >355 mm	81 (37)	39 (52)	56 (89)
C: Depth of straw penetration (spring and fall 1982)[c]	90 (84)	89 (71)	90 (155)
D: Angle of straw penetration (spring and fall 1982)[c]	94 (31)	95 (38)	94 (69)
A plus C (spring 1982)	100 (35)	95 (19)	98 (54)
C plus D (fall 1982)	97 (32)	91 (32)	94 (64)

[a] Two additional bass could not be sexed by this method.

[b] Eleven additional bass could not be sexed by this method.

[c] Two additional bass could not be sexed by this method.

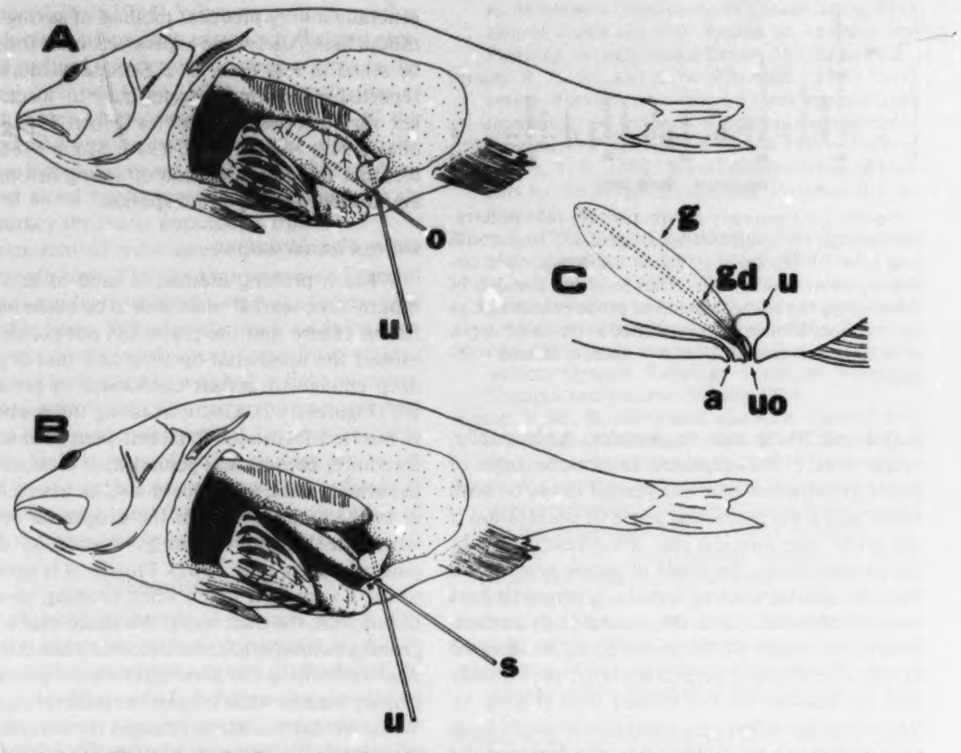

FIGURE 1.—(A) Illustration of a female largemouth bass with sexing probes inserted into the oviduct (o) and urinary bladder (u). (B) Illustration of male largemouth bass with probes inserted into the sperm duct (s) and urinary bladder (u). Note that the angle of probe penetration into the genital duct of both male and female fish is at an oblique angle to the main body axis whereas penetration into the bladder is generally perpendicular. Note also that the sexing probe penetrates much deeper into the oviduct than it does into the sperm duct (the lumen of the oviduct being wider), whereas it penetrates to a similar depth into the urinary bladder of both sexes. In practice, because of the differential ease of penetrating the various ducts, our 1-mm-diameter probe would typically pass deeply into the oviduct of females, but would usually only enter the urinary bladder of males. (C) Schematic drawing of the urogenital region of largemouth bass: anus (a); gonad (g); urinary bladder (u); genital duct (gd: solid lines = male duct, dashed lines = female extension); urogenital opening (uo).

though the method may be successful for experienced people who continually examine largemouth bass (Parker 1971), we feel that the criterion "round or oval scaleless area around the urogenital opening" is too subjective and variable. The shape of the scaleless area may be influenced by seasonal gonad enlargement or stomach fullness, or spatial attitude during examination. Manns and Whiteside (1980) found the accuracy of sexing their small sample of largemouth bass increased to 84% when they excluded fish exhibiting distended or concave abdomens. We did not attempt to duplicate this effort as we felt the original technique was already overly subjective.

Probing the urogenital opening to measure depth or angle of penetration proved an effective way to sex largemouth bass; about the same levels of accuracy were achieved for males and females (Table 1). Depth of penetration ranged from 0 to 40 mm for males (83% of probes were within the range of 0–20 mm) and from 0 to 60 mm for females (82% of probes were within the range of 30–60 mm; Figure 2). Therefore, we adopted 25 mm as the cutoff depth (Figure 2) for sexing largemouth bass with our 1-mm probe (<25 mm = male; ≥25 mm = female). Subsequently no attempt to insert the probe to the furthest depth was needed to sex most females accurately.

Sexing bass with a 0.8-mm probe, Glass et al. (1962) noted penetration depths of 8–13 mm for

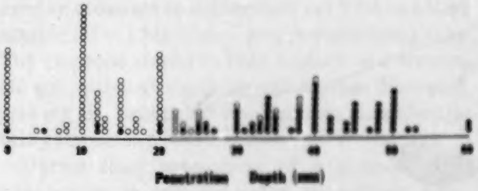

FIGURE 2.—Frequency distribution of probe penetration through the urogenital openings of 155 largemouth bass subsequently sexed by gonad inspection; open circles represent males, solid circles represent females. In determining the sex with depth of probe penetration as the criterion, 25 mm was established as the cutoff depth separating the sexes (<25 mm = male, ≥25 mm = female).

males and 30–58 mm for females. Additionally, Glass et al. (1962) reported an oblique angle of probe penetration into the genital ducts of both sexes, and a perpendicular angle of penetration if the probe was directed into the urinary duct. In the present study, the angle of probe penetration into the genital duct of female largemouth bass was typically oblique to the ventral body surface, whereas in males (if the probe could be inserted at all) it penetrated perpendicularly, presumably into the bladder via the urinary duct (Figure 1). We believe the differences noted here between these two studies can be explained by the fact that the probe used by Glass et al. (1962) was 0.2 mm smaller in diameter than that used in the present study and thus allowed entry into the sperm duct of some male fish.

Concurrent use of several sexing methods produced the highest levels of accuracy (Table 1). For instance, the highest rate of sexing success (98%) occurred during the spring, when fish were sexed by inspection for a urogenital papilla plus probing of the urogenital opening. During the fall, a combination of probing depth and angle of penetration resulted in an overall accuracy of 94%. Additionally, some females began to exhibit a reddish urogenital papilla by late fall.

Problems that hampered probe insertion into largemouth bass were (1) insufficient polishing of the probe tip, which caused it to snag on the genital duct wall, (2) distension of the stomach with food so that the genital duct was distorted, (3) occasional occurrence of underdeveloped ovaries, and (4) presence of a thin layer of skin, which closes off the oviduct during late summer (Braungart 1951; Glass et al. 1962) and which had to be punctured before insertion was possible. Despite occasional problems with probing, we feel it was generally a very practical method of sexing largemouth bass. After some experience, most fish could be sexed in less than 20 s. Several authors have reported using similar techniques to successfully sex various other fish species (Moen 1959; Glass et al. 1962; Norton et al. 1976; Mohr 1982). We feel that this rapid method of sexing fish may be applicable to many more species.

Other Considerations

When a probing method is used to sex largemouth bass, several trials should be made on each fish to ensure that the probe has not accidentally missed the urogenital opening and that it passes deep enough to permit confidence in predicting sex (Figures 1, 2). Gently rotating the probe as it is inserted facilitates maximal penetration. Furthermore, because the genital duct is anterior to the urinary duct, the probe should be inserted closer to the anterior wall of the urogenital opening when the separate openings cannot be distinguished (Glass et al. 1962). Finally, it is necessary to use a very light touch when probing, so as not to puncture the duct walls. We noted that a light-probing technique allowed us to feel when the probe was confronting the somewhat elastic gonadal or urinary bladder walls long before puncturing them. While we did not assess changes in mortality and reproductive success associated with probe punctures, we did recapture numerous largemouth bass that we had previously sexed. Furthermore, Ross (1981) noted no mortality or effects on growth and behavior after catheterization of the genital tract and gonads of female wrasses (*Thalassoma duperrey*). Shehadeh et al. (1973) noted no abnormal oocyte development associated with weekly catheterization.

Catheterization is commonly used to extract gametes and gonadal tissue in order to monitor reproductive status and to identify the sex of sexually monomorphic fishes (Stevens 1966; Chen et al. 1969; Shehadeh et al. 1973; Ross 1981, 1984). Although advanced oocytes and mature ova can often be identified macroscopically, microscopic examination of extracted tissue is required during certain reproductive phases. Additionally, the catheter (usually a glass hematocrit tube or polyethylene tubule) must be disposable or cleaned between uses. Catheterization, therefore, can be more time consuming than the probing technique in identifying the sex of fishes. Catheterization is a very effective technique to extract oocytes and ova, but difficulties in introducing the catheter into male fish and in obtaining sperm samples have been noted (Ross 1984). When no gametes or other reproductive tissues are extracted, the catheterization technique relies on the same criteria as the probing method (Ross 1984). It appears that at certain times of the year a combination of the catheter and probing methods would allow many fishes to be accurately sexed. However, the probing method alone might provide high enough levels of accuracy for many population studies.

A hierarchy of techniques exists for sexing live largemouth bass. The simultaneous use of several methods can increase the percentage of correct sex determinations. When predictions made by several methods are inconsistent, input from various sexing methods must be weighed in light of known seasonal variations (e.g., stripping gametes and presence of a urogenital papilla). It seems probable that the highest rates of sexing success can be attained in the spring when sexual dimorphism is most pronounced and the greatest combination of sexing methods can be used simultaneously.

Acknowledgments

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TECHNICAL NOTES

An Inexpensive Demand Feeder for Cage-Reared Tilapia

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Abstract.—An inexpensive demand feeder was evaluated for rearing blue tilapia (*Tilapia aurea*) in floating cages. The feeder was tested in a farm pond over a 10-week period to estimate labor savings and to compare production of fish fed by demand feeder and fish fed by hand to satiation. Fish fed by demand feeders gained an average of 72% more weight than hand-fed fish. Feed conversion and survival rates were not significantly different between the two feeding methods.

The usefulness of cages to raise fish has been demonstrated in watershed farm ponds as well as in large reservoir impoundments (Schmittou 1970). However, the time and expense required for daily feeding of caged fish can be excessive, especially if boats are required for access to the cages. Coche (1978) suggested that demand feeders could be used to reduce labor costs in feeding fish. Frequent feeding may promote the growth of stomachless fish such as common carp (*Cyprinus carpio*) and *Tilapia* spp. (Lovell 1980) as well as channel catfish (*Ictalurus punctatus*) (Greenland and Gill 1981). In this study, a demand feeder constructed of commonly available materials was designed for use with fish cages. This feeder was tested in a farm pond over a 10-week period to estimate labor savings and to compare production of fish fed by demand feeder to that of fish fed by hand to satiation.

The main components of the feeder are an 18.9-L plastic bucket with lid, a funnel tube, and an activating mechanism (Figure 1). The feeder is designed to be placed on top of a solid cage lid, with the activating mechanism extending into the water through a hole cut in the lid. The plastic bucket lid contains a gasket to prevent any water from entering and contacting the feed. The funnel tube is a 7.6- to 6.4-cm polyvinyl chloride (PVC) reducer seated in an opening cut in the bottom of the bucket. Screws hold the funnel tube in place during platform adjustments and cage inspections. Feed drops by gravity onto an adjustable 9-cm-diameter circular tin feed platform positioned below the funnel tube and above water level on a free-swinging activator rod. When the rod is not activated by fish, feed pellets are retained on the platform if the gap (distance) between the platform and the funnel tube is adjusted to be sufficiently small. The activator rod is a threaded steel rod about 46 cm long with a flattened upper end. The rod is suspended from the funnel tube on a V-shaped stainless steel wire that holds the rod in the center of the funnel tube and facilitates the flow of feed pellets through the funnel. A piece of plastic screen folded in half is attached to the bottom of the activator rod. This screen increases the contact area while permitting water to flow through the mesh without triggering the release of feed pellets. A few feed pellets soaked in clear resin, dried, and placed in the fold of each screen attract the fish. As the fish attempt to reach the coated pellets, the screen and rod are moved, causing the pellets to drop from the edge of the feed platform. During design trials, this screen appeared to help train the fish to use the demand feeder more quickly than a feeder without an activator screen.

Tilapia grow well in cages (Pagan 1970) and were used to evaluate the demand feeder. Six 1.0-m^3 floating fish cages were each stocked with 300 blue tilapia (*Tilapia aurea*) fingerlings. Mean stocking weights (Table 1) and proportions of male to female fish were not significantly different among cages. Fish in three cages were hand-fed once daily to satiation in a 20-min period, 6 d/week. Fish in the other three cages fed from demand feeders. Feed in both treatments consisted of a catfish diet in the form of 32% crude protein floating pellets.

Fish fed by demand feeders gained an average of 72% more weight than hand-fed fish over the 11-week period (Table 1). Feed conversions and survival rates were not significantly different between the two treatments although the mean conversion in the demand feeder treatment had a relatively large standard deviation.

The gap between the feed platform and the bottom of the funnel tube (Figure 1) was 15–17 mm for 7.9-mm-diameter pellets. A wider gap caused more feed pellets to fall than the fish would eat when the activator rod was moved. Feed in the demand feeders stayed dry, even under adverse

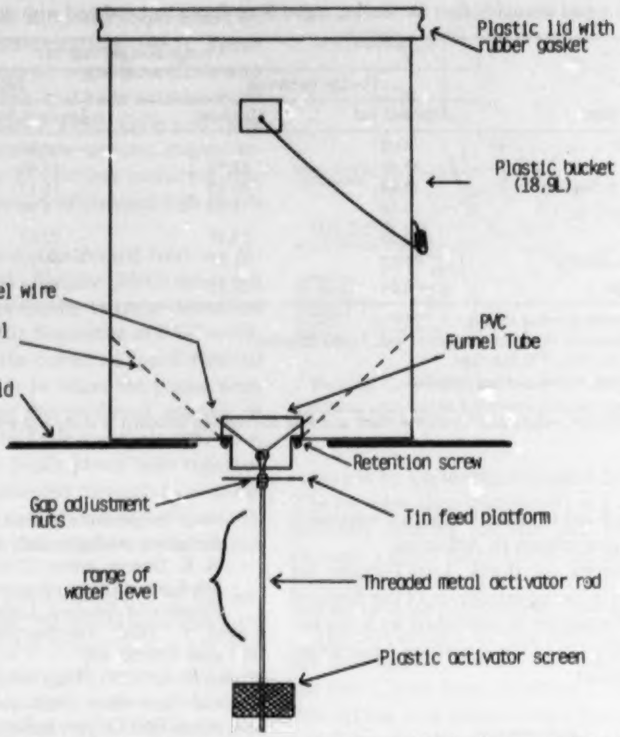
Plastic lid with rubber gasket
Plastic bucket (18.9L)
Bent steel wire
Funnel
Cage lid
PVC Funnel Tube
Retention screw
Gap adjustment nuts
Tin feed platform
range of water level
Threaded metal activator rod
Plastic activator screen

FIGURE 1.—Schematic diagram (not to scale) of a demand feeder for use on fish cages.

rain and wind conditions. High wave action occasionally activated the rod and caused excess feed pellets to fall; this effect was reduced by installation of styrofoam floats and a small-mesh feeding ring on each cage.

Reducing the gap between the feed platform and the funnel tube may result in improved and less variable feed conversions, but fish weight gains may be lower. A larger capacity feed bucket would permit less frequent filling of the demand feeder or a larger biomass of fish to be fed. The addition of a sloped bottom (Figure 1) is suggested to prevent accumulations of unused pellets in the bucket.

This preliminary study indicates that demand feeders can reduce labor costs and increase fish weight gains (Table 2). A larger profit margin per cage would be expected in large operations. The criteria used in calculating the estimated labor costs for the demand feeder operations listed in Table 2 included 2.5 visits per week at 0.5 h travel time per trip, and 4 min feeding time per cage. The criteria used for the hand-feeding operation were seven visits per week, 0.5 h travel time, and 2 min feeding time per cage.

Based on these results, use of demand feeders could enable caged blue tilapia to approach a market size of 227 g within a 5-month growing season.

TABLE 1.—Production data for caged blue tilapias fed 11 weeks by hand or from demand feeders. Data are means ± SDs for three cage groups. When means differ significantly between treatments, an asterisk denotes the larger value (one-way analysis of variance; $P = 0.05^*$).

Treatment	Initial weight (g)	Total weight gain (g)	Feed conversion (g/g)[a]	Survival (%)
Hand-fed	8.41±0.66	56.67±2.48	1.58±0.09	96.8±2.59
Demand-fed	8.23±1.24	97.70±2.63*	2.30±0.27	94.2±1.75

[a] Feed conversion = (dry weight of feed fed)/(wet weight gain of fish).

TABLE 2.—Estimated annual budget for feeding caged blue tilapia by hand and with demand feeders.[a]

Budget item	Average cost per cage ($)			
	10-cage operation		100-cage operation	
	Demand-fed	Hand-fed	Demand-fed	Hand-fed
Equipment[b]	4.00		4.00	
Feed cost[c]	47.40	18.93	47.40	18.93
Labor cost in feeding[d]	25.68	50.16	15.17	23.61
Fish cost[e]	5.50	5.50	5.50	5.50
Total costs	82.58	74.59	72.67	48.04
Value of production[f]	91.22	54.37	91.22	54.37
Profit margin	+8.64	−20.22	+18.55	+6.33

[a] Based on a 22-week growing season.

[b] Materials and labor for feeder construction with 3-year lifetime.

[c] Based on $0.35/kg feed, 300 fish/cage.

[d] At $4.00/h, no gas or boat charges included.

[e] At $2.20/kg.

[f] Based on stocking rate, weight gains, survival rates, and feed conversions obtained in study and a farm price estimate of $1.65/kg whole tilapia.

Blue tilapia hand-fed in cages would not approach market size in one season in Arkansas.

Acknowledgments.—I thank Les Torrans for technical advice in the construction of the demand feeder. This information is published with the approval of the Director of the Arkansas Agriculture Experiment Station.

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The Progressive Fish-Culturist 48:228–229, 1986

A Technique for Hatching Eggs of Crevice-Spawning Minnows

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Abstract.—Spotfin shiners (*Notropis spilopterus*) willingly spawned in simulated crevices formed by spaced plastic rectangles bolted together in a stack. The plastic plates can be separated and transferred, with the adhesive eggs, to incubation aquaria, where large numbers of fry can be hatched for experimental or aquacultural purposes.

To assess the feasibility of culturing spotfin shiners (*Notropis spilopterus*) in power plant effluents, it was necessary to find a simple method for collecting and incubating large numbers of eggs. This paper describes a simple technique for collecting and hatching large numbers of eggs of the spotfin shiner, a crevice-spawner (Pflieger 1965), with use of artificial substrates and an egg-transfer method.

Other crevice-spawners include the steelcolor shiner (*Notropis whipplei*), the blacktail shiner (*Notropis venustus*), and the whitetail shiner (*Notropis galacturus*) (Pflieger 1975) and some other species belonging to the subgenus *Cyprinella* (W. F. Gale, personal communication, 1985). These fish are valuable as bioassay organisms (Gale and Gale 1976) and forage fish, and have a commercial potential as baitfish (Stone 1940; Gale and Gale 1977). In nature, crevice-spawners commonly deposit eggs in a variety of crevices including tree roots and the undersides of submerged logs (Scott and Crossman 1973).

Spawning plates were constructed from an ⅛-in-thick, gray polyvinyl chloride (PVC) sheet cut into 4 × 6-in rectangles similar to those described by Gale and Gale (1976). Segments of PVC welding rod were glued in the corners of each plate to maintain a spacing of ⅛ in when the plates were stacked, approximating the preferred spacing of 0.06–0.12 in reported by Gale and Gale (1976). A stack consisted of five or six plates held together by a 3-in brass bolt inserted through a ¼-in-diameter hole and held by a brass nut (Figure 1). Brass was used because it is nontoxic and does not rust.

After adult captive fish had deposited the adhesive eggs on the plates, the plates were carefully separated and incubated in 10-gal aquariums. Egg plates were suspended vertically at mid-depth in the aquarium by sliding them onto a 17-in-long hooked ¼-in-diameter brass rod. A 3-in section of ⅜-in-diameter tubing joined a second brass hook to the rod and provided support for up to 13 plates. Friction between the brass rods and the tubing securely held the hatching assembly together. The individual plates were separated on the rod by 1-in segments of ½-in-diameter tubing that maintained spacing between plates to prevent egg damage (Figure 2). Airstones were used to oxygenate the water and prevent silt from settling on the eggs. The rods, plates, and airstones were removed after the eggs hatched. Once the fry became active, water was removed by siphoning through a fine-mesh screen. The fry were then transferred to a rearing tank by carefully pouring the fish and remaining water through a small notch cut in the plastic rim of the aquarium. Hatching time and survivability were similar to those described by Gale and Gale (1977).

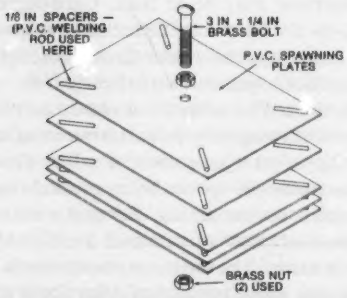

FIGURE 1.—Exploded view of a spawning-plate stack.

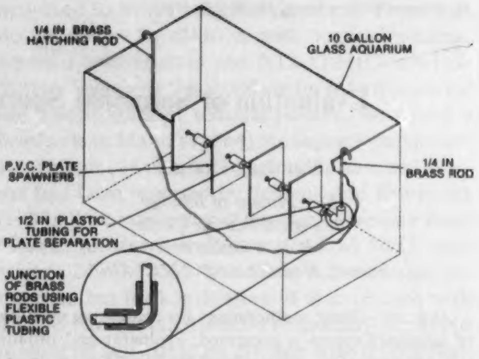
1/4 IN BRASS HATCHING ROD
10 GALLON GLASS AQUARIUM
P.V.C. PLATE SPAWNERS
1/4 IN BRASS ROD
1/2 IN PLASTIC TUBING FOR PLATE SEPARATION
JUNCTION OF BRASS RODS USING FLEXIBLE PLASTIC TUBING

FIGURE 2.—Cutaway view of complete aquarium hatching apparatus for crevice-spawning minnows.

The advantages of this method are: (1) plates are manageable, sturdy, and readily accepted by the fish; (2) the brass hatching rod joined by flexible tubing to a second brass hook facilitates the handling of plates and provides the support needed without slipping apart; (3) glass aquariums are inexpensive, allow observation of eggs and fry, and are lightweight so the culturist can transfer fry to rearing tanks or ponds as needed. The technique described was evolved over several months and has proved to be very convenient and efficient.

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The Progressive Fish-Culturist 48:230–232, 1986

Evaluation of Salmonid Sperm Motility for Cryopreservation

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Abstract.—Basic methodology for evaluating motility of salmonid sperm is presented. Problems and pitfalls that are commonly encountered are identified, and suggestions are made for avoiding them. Sperm dilution in preparation for cryopreservation is discussed.

The simplest and most reliable indicator of semen (milt) quality is sperm motility. This criterion is commonly used in the selection of milt, both for immediate insemination and for preservation by freezing. In the current state of the art, the number of salmonid sperm that survive freezing is too small to make cryopreservation a practical procedure (Scott and Baynes 1980). In this paper, guidelines for the evaluation of salmonid sperm motility are given and a procedure that resulted in partial recovery of motility after freezing is described.

The sperm of salmonids, like those of most families of fish, are immotile in seminal plasma until they are activated by contact with ovarian fluid. Their mobilization is not dependent on the presence of eggs. Strained ovarian fluid, cleared by centrifugation at high speed, will activate trout sperm, and 0.12 M (0.7%) NaCl is equally effective (Terner and Korsh 1963). A distinctive feature of salmonid sperm is the very short duration of their motility; trout sperm may be motile as briefly as 30 s and Atlantic salmon (*Salmo salar*) sperm motility may last only 1–2 min. Viewing with a microscope one can see a sudden burst of motility immediately after dilution with the NaCl solution. The vigorous motility subsides rapidly but unevenly. Because the milt must be diluted and the slide placed under the microscope, the highly motile stage may be missed by the time the slide is reviewed. A minimum delay between dilution and observation is essential. The interval between milt collection and testing is not critical; kept in a test tube surrounded by crushed ice, the milt sample will remain viable for several days.

The following procedure is recommended for testing sperm motility. The microscope is prefocussed on an empty slide. A drop of 0.7% NaCl is placed on the slide and a smaller drop of milt is placed next to it; a saline : milt ratio of 2:1 is recommended. If the drops run together at this stage, begin again with fresh drops of milt and saline. The drops are mixed by placing a coverslip over them and, after the slide is focussed under the low-power objective, the sperm are viewed under high power. If too much time is taken adjusting the focus, only a few sperm may be seen still moving sluggishly; however, with the microscope now in focus, the procedure can be repeated more rapidly. A teaming, flickering motion can be observed in dense suspensions, and a greater dilution will allow the tightly packed cells to separate, so that individual sperm can be seen racing across the field. Stationary vibration or slow circular motion by fresh specimens within the first 30–60 s after dilution indicates poor quality. In a sample that has been subjected to freezing and thawing, slow motion is a welcome indicator of survival. Passive streaming may occur due to currents under the coverslip that are caused by incomplete mixing of milt and saline. Groups of sperm drifting in the same direction should not be mistaken for evidence of motility. If streaming interferes with the evaluation, prepare another slide with smaller drops. If, because of high sperm density, it is necessary to dilute a small drop of milt with a large drop of saline, streaming can be minimized if the milt is placed on the coverslip as a hanging drop and lowered into the saline on the slide.

If vigorous motility cannot be observed in repeated attempts, either the quality of the sperm or the operator may be at fault. Curdling of the milt in ovarian fluid sometimes occurs; sperm in ovarian fluid inspected under the microscope may aggregate (stick together head to head). If the sperm can be activated in saline, but clump in ovarian fluid, the clumping may be an immunological response. Apparent aggregation in saline also may be due to immotile sperm being pushed together into clusters by streaming. Several evaluations should be conducted before a final decision of poor motility is made. The most common error is missing the active phase because of delays between the time of mixing the drops on the slide and focusing the microscope. Because the observation must be made within 30–60 s after dilution, one should always work with a stopwatch, starting it at the moment of mixing. When an operator, consistently using the same technique on several males, finds specimens of poor motility among a number of motile ones, the negative findings may be considered more reliable. Sperm motility varies in vigor and duration not only among males, but also within an individual male depending on his ripeness. Activity is best at the height of the breeding season and declines as the season progresses.

The motility of salmonid sperm can be intensified and prolonged by adding 3-isobutyl-1-methylxanthine to the diluent (Benau and Terner 1980). Methylxanthines (e.g., caffeine) inhibit cyclic AMP-phosphodiesterase. This reagent can be useful in testing milt samples of marginal quality and during the decline of activity near the end of the season.

Water should not be used to activate salmonid sperm. In older manuals "wet" and "dry" methods of insemination are described. In the wet method, a little water is scooped up with the egg pan before the first female trout is stripped. Because insemination in the presence of water appeared to be closer to natural conditions, the wet method was more popular. It is well known that water hardens the shell (chorion) and closes the micropyle, the opening through which the sperm enters the egg. Spermatozoa are much more vulnerable than eggs. In a hypotonic environment, osmotic pressure forces water across the sperm membrane and causes irreparable damage. Even though it may be possible, by adding water cautiously, to achieve sufficient dilution of the milt for motility to appear before a damaging level of hypotonicity is reached, this is not recommended in field practice. In nature, eggs are fertilized not with the aid of, but in spite of, the presence of water. Both eggs and sperm are protected by their isotonic fluids.

In our previous studies, we had been working with mammalian sperm, which are continuously motile, so we found the unique features of salmonid spermatozoa intriguing. Why are they immotile in seminal fluid and why, after activation, can they sustain their activity for only a short time? To test whether or not immotility was merely due to overcrowding and lack of oxygen in the densely packed milt, we diluted milt with seminal plasma (from the same source) that had been centrifuged at high speed and aerated, and with seminal plasma from another trout, certrifuged and heat-inactivated to avoid immune reactions. There was no activation by dilution with seminal plasma, whereas ovarian fluid and 0.12 M NaCl were effective. Searching the literature for information on the composition of seminal plasma, we found a brief note in Mann's (1964) monograph that a high K^+ content (24 meq/L) in salmonid seminal plasma had been reported by Schlenk and Kahmann (1938). We confirmed that dilution of rainbow trout (*Salmo gairdneri*) milt with 0.12 M NaCl containing 24 mM KCl (K^+ saline) did not cause activation, but further dilution of this mixture with 0.12 M NaCl elicited a burst of motility. To avoid premature activation by dilution with cryoprotective media, the addition of KCl before freezing was suggested (Benau and Terner 1980; Legendre and Billard 1980). Using glycerol in 5–8% concentrations in 0.12 M NaCl, which contained 24 mM KCl as the cryoprotective medium, we froze a number of samples of rainbow trout and Atlantic salmon milt in liquid nitrogen. The thawed samples were agitated in K^+ saline for at least 5 min at 5–10°C and were tested for motility by dilution with 0.12 M NaCl. They remained immotile for about 2 min; then a slow vibratory motion with some progressive movement appeared and spread through the whole field of vision. Although only marginal motility was recovered, these observations provided evidence of the physical survival of large numbers of sperm and demonstrated the need for a period of recovery after thawing. Because the metabolism of salmonid sperm is predominantly aerobic, recovery in an aerated immobilizing medium may be useful in cryopreservation programs.

Acknowledgments.—I thank the staffs of national and state fish hatcheries in the New England region for their generous cooperation.

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Symbols and Abbreviations

The following symbols and abbreviations, as well as others approved for the Système International d'Unités (SI), are used in this journal without definition. Any others must be defined in the text at first mention, as well as in the captions or footnotes of tables and in figures or figure captions.

Prefixes

kilo (10^3)	k
milli (10^{-3})	m
micro (10^{-6})	μ

Time and Temperature

day	d
degrees Celsius ([°F − 32]/1.8)	°C
degrees Fahrenheit ([1.8 × °C] + 32)	°F
hour (spell out for diel time)	h
minute	min
second	s

Spell out year, month, and week.

Weights and Measures (Metric)

centimeter (0.394 in)	cm
gram (0.0353 oz)	g
kilogram (2.20 lb)	kg
kilometer (0.622 mi)	km
liter (0.264 gal, 1.06 qt) (exception to SI)	L
meter (1.09 yd, 3.28 ft, 39.4 in)	m

Weights and Measures (English)

cubic feet per second (0.0283 m^3/s)	ft^3/s
foot (30.5 cm)	ft
gallon (3.79 L)	gal
inch (2.54 cm)	in
mile (1.61 km)	mi
ounce (28.4 g)	oz
pound (0.454 kg, 454 g)	lb
quart (0.946 L)	qt
yard (0.914 m, 91.4 cm)	yd

Spell out acre (0.405 hectare), hectare (2.47 acres), ton (2,000 lb, 907 kg), and tonne (1,000 kg, 2,200 lb).

Mathematics and Statistics

all standard mathematical signs, symbols, and abbreviations	
base of natural logarithm	e
common test statistics (F, t, etc.)	
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
degree (angular or temperature)	°
degrees of freedom	df
logarithm (specify base)	log
minute (angular)	′
not significant	NS
percent	%
probability	P
sample size	N
second (angular)	″
standard deviation	SD
standard error	SE

Physics and Chemistry

all atomic symbols	
alternating current	AC
ampere	A
British thermal unit (1,055 J)	Btu
chemical acronyms listed in Webster's dictionaries (DDT, EDTA, etc.)	
direct current	DC
footcandle (0.0929 lx)	fc
hertz	Hz
hydrogen ion activity (negative log of)	pH
joule (0.239 gram-calories, 0.000948 Btu)	J
lux (10.8 fc)	lx
molar	M
mole	mol
ohm	Ω
ortho	*o*
para	*p*
pascal	Pa
parts per million (per 10^6) (in the metric system, use mg/L, mg/kg, etc.)	ppm
parts per thousand (per 10^3)	ppt, ‰
siemens (=mho, Ω^{-1})	S
volt	V
watt	W

Calories should be converted to joules in the metric system.

General (Some Are Restricted)

compass directions (maps and coordinates): east	E
north	N
south	S
west	W
District of Columbia	D.C.
et alii	et al.
et cetera	etc.
for example	e.g.,
months (tables, figures): first three letters (Feb, Jun, etc.)	
sex (tables, figures, hybrid crosses): female	♀
male	♂
that is	i.e.,
Union of Soviet Socialist Republics	USSR
United States (adjective)	U.S.
United States of America (noun)	USA

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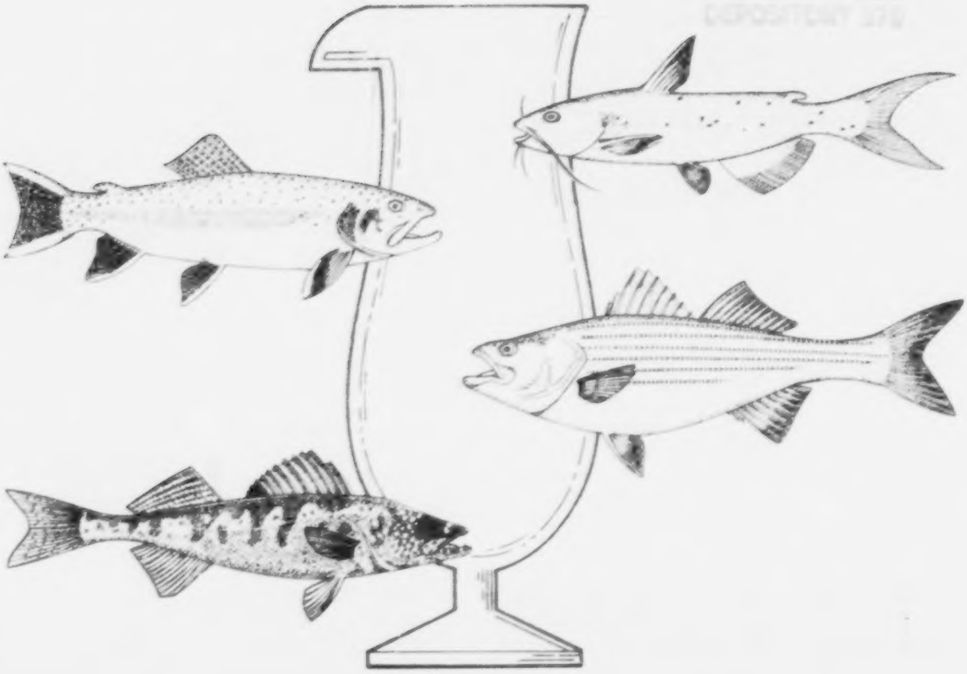

American Fisheries Society

in cooperation with

United States
Department of the Interior
Fish and Wildlife Service

Volume 48
Number 4
October 1986

I 49.35:

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Optimum Ratio of Dietary Protein to Energy for Red Crayfish (*Procambarus clarkii*)

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Abstract.—Six experimental diets were formulated with protein-to-energy (P:E) ratios ranging from 57 to 160 mg protein/kcal. The diets, which were fed to juvenile red crayfish, contained either 20, 30, or 40% crude protein and 2.5 or 3.5 kcal of total energy per gram of diet. As dietary energy increased, growth of crayfish fed the 30% protein diets decreased, but growth of crayfish fed the 40% protein high-energy diets increased. In general, the percentage of body protein (wet weight) decreased as dietary energy increased. Crayfish fed low-energy (low-fat) diets had lower percentages of body fat. Also, body fat tended to decrease as dietary protein increased. Best growth and protein deposition were observed in crayfish fed a diet with a P:E ratio of 120 mg protein/kcal; this diet contained 30% crude protein and 2.5 kcal/g dietary energy.

Both red crayfish and the white crayfish (*Procambarus acutus acutus*) are important commercial food species, and their culture is a multimillion-dollar industry in the United States. Crayfish aquaculture is practiced in approximately 40,000 hectares in Louisiana and 5,000 hectares in Texas with smaller areas under culture in other states.

Formulated feeds are not normally used in crayfish culture; rather a detritus-based ecosystem is established to provide food. A shortage of available food in such a system is responsible for a major problem in crayfish farming, that of stunted (submarketable) individuals (Avault et al. 1974). The prevention of stunting in crayfish has been aided by supplemental feeding or by feeding a complete feed designed for other species (Huner and Barr 1981). Although feeds formulated for other aquatic animals have generally increased crayfish production, economic considerations have precluded commercial use. Feeds specifically formulated to meet the nutritional requirements of crayfish should be more economical than those previously used. In addition, feeding a nutritionally balanced feed to crayfish should increase yield and may contribute to the production of a year-round crop. Currently, crayfish are generally available from November to May only.

Essentially no information exists concerning the nutritional requirements of crayfish. Based on a study by Huner and Meyers (1979) the protein requirement for the red crayfish was estimated to be 20 to 30% of the diet. However, no study has considered the effect of dietary ratios of protein to energy (P:E) on the growth of crayfish. The objective of this study was to evaluate the effect of dietary P:E ratios on the growth and chemical composition of red crayfish.

Methods

Diets and experimental design.—An 8-week study was conducted to determine the optimum dietary P:E ratio for red crayfish. Six experimental diets were formulated; casein and gelatin were used as protein sources (Table 1). Fish oil and dextrin levels were varied to give the desired level of dietary energy. Since neither digestible nor metabolizable energy values are available for crayfish, dietary energy values were calculated (based on proximate analysis of diets) from standard vertebrate physiological fuel values of 4 kcal/g for protein or carbohydrate and 9 kcal/g for lipid (Maynard et al. 1979). A diet similar to the one used in the present experiment has been routinely used for various finfish studies in our laboratory as well as in other laboratories.

Dietary P:E ratios ranged from 57 to 160 mg protein/kcal in diets that contained either 20, 30, or 40% crude protein at energy levels of 2.5 or 3.5 kcal total energy per gram of diet. All experimental diets were prepared in our laboratory by mechanically mixing dry ingredients in a V-mixer for 30 min and then adding oil to the dry mix and dispersing it with a Hobart mixer. After the oil was dispersed, approximately 300 mL of distilled water per kilogram of dry diet was added to give a consistency that would facilitate pelleting. The dough-like mixture was then pelletized in a meat grinder to form 2-mm-diameter pellets. The pellets were air dried to a moisture level of approximately 10%. The use of a binder (carboxymethyl cellulose) resulted in diets that were water stable for several hours. Prior to feeding, all diets were broken into particle sizes that were easily consumed by all crayfish. Particle size was increased as the crayfish grew.

Ninety-six crayfish were individually maintained in 10-cm-diameter cylindrical polyvinyl chloride (PVC) containers. Four containers were placed into each of 24 50 × 25 × 25-cm glass aquaria. Four aquaria (16 crayfish) were used for each diet treatment. The number of crayfish used in the present study was limited due to space restrictions. A recirculating water system was used in which water flowed through individual aquaria and returned to a 120-L settling chamber via a common drain. Water passed from the settling chamber into a submerged sand and gravel biofilter before being returned to each aquarium. Each aquarium was equipped with two airstones and air was supplied by a low-pressure, high-volume blower.

Juvenile crayfish obtained from commercial sources were acclimated to experimental conditions. After acclimation, uniform-size crayfish of 33–36 mm total length were selected and stocked into the individual containers. Each crayfish was fed a casein–gelatin conditioning diet which was similar to diet 3 (Table 1) except the energy level was approximately 3.0 kcal/g. This diet was fed during the 2 weeks prior to the start of the experiment.

Individual weights and total lengths were recorded at the beginning of the experiment and every 2 weeks thereafter. Crayfish were fed to excess twice daily. Feces and uneaten feed were siphoned from each aquarium daily. Water was added to the biofilter as needed to replace losses from siphoning. Aquaria were checked two to three times daily for molts, which were removed to prevent consumption of additional sources of protein and energy. This method appeared to work well in that a high percentage (estimated to be 90% or better) of molts were removed. Dead crayfish were replaced during the first week of the experiment but not afterwards.

Water quality.—Dissolved oxygen and water temperature were measured three or four times each week in randomly selected aquaria with a YSI model-51B oxygen meter and temperature probe (YSI, Yellowsprings, Ohio). Total ammonia, nitrite, pH and calcium hardness were determined weekly with a Hach DR-EL/2 spectrophotometer (Hach Chemical Company, Ames, Iowa). Calcium hardness was maintained at 50–100 mg/L through the addition of $CaCl_2$. This level of hardness was in the range considered adequate for normal growth of crayfish (de al Bretonne et al. 1970).

Sample collection and analysis.—Upon termination of the experiment, all crayfish from each aquarium were collected and frozen at −15°C for subsequent chemical analyses of whole bodies. Crayfish were taken in all phases of molt; however, more than 80% were in intermolt stages. The inclusion of crayfish from stages other than intermolt may have affected composition data. Six to eight whole crayfish were pooled and homogenized with a Polytron homogenizer (Brinkmann Instruments, Westburn, New York). Each analysis was conducted in duplicate on two pooled samples per diet treatment.

Dry matter and ash were determined by means of AOAC (1980) methods. Whole body protein

TABLE 1.—Composition and analysis (dry weight basis) of experimental diets fed to red crayfish.

Measure	Diet number					
	1	2	3	4	5	6
Diet ingredient (%)						
Casein[a]	18.7	18.7	28.1	28.1	37.4	37.4
Gelatin[a]	3.0	3.0	4.5	4.5	6.0	6.0
Dextrin[a]	35.2	42.2	24.3	31.3	13.5	20.6
Menhaden fish oil[b]	2.0	10.0	2.0	10.0	2.0	10.0
Carboxymethyl-cellulose[a]	2.0	2.0	2.0	2.0	2.0	2.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[c]	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix[c]	5.6	5.6	5.6	5.6	5.6	5.6
Cellulose[a]	32.5	17.5	32.5	17.5	32.5	17.4
Diet analysis						
Protein (%)	20	20	30	30	40	40
Energy (kcal/g)	2.5	3.5	2.5	3.5	2.5	3.5
Protein-to-energy ratio (mg protein/kcal)	80	57	120	86	160	114

[a] U.S. Biochemical Corporation, Cleveland, Ohio.

[b] Zapata Haynie Corporation, Cameron, Louisiana.

[c] Composition as given by Robinson and Rawles (1985).

was determined by the macro-Kjeldahl method (AOAC 1980). Whole body lipid was determined by chloroform–methanol extraction (Folch et al. 1957).

Statistical analysis.—Growth and body composition data associated with different dietary P : E ratios were compared by the Statistical Analysis System, SAS-79 (Helwig and Council 1979), following the general linear models procedure. Duncan's multiple-range test was used to determine statistical differences between treatment means (Steel and Torrie 1960). Results were considered significant at the 0.05 level.

Results and Discussion

Water Quality

Ammonia (NH_3-N) ranged from 0.03 to 0.46 mg/L and nitrite (NO_2-N) from 0.07 to 0.83 mg/L. These levels remained low for most of the experiment but there were occasional high pulses of each. Dissolved oxygen ranged from 5.5 to 7.8 mg/L and pH from 6.5 to 8.0. Except for a 2-week period in which the temperature dropped to 22°C, the temperature was 25 to 27°C. The low water temperature appeared to reduce feed consumption of all crayfish as evidenced by the amount of uneaten feed remaining in all cages. Fluctuations in water quality may have contributed to the within-treatment variation observed during the experiment but the actual effects were not determined.

Growth and body composition.—Crayfish fed diet 3 (P:E = 120; 30% protein; 2.5 kcal energy/g) had the greatest percentage increase in weight; however, the increase was not significantly different from those of crayfish fed diets 1, 2, or 6 (Table 2). As dietary energy increased, there was a slight (but not significant) reduction in percent weight increase in crayfish fed diets containing 20% protein, a significant reduction in those fed diets containing 30% protein, and a significant increase in those fed diets containing 40% protein. It is generally accepted that many animals eat to satisfy an energy need; this has been documented for certain finfish (NRC 1983). The observed growth depression of crayfish fed the higher energy diets at 20 and 30% protein levels may have been related to a reduction in feed consumption resulting in insufficient protein intake. The reason that the growth depression was not as severe in crayfish fed the 20% protein diet is not known. Even if consumption was decreased at higher energy levels, 40% dietary protein was probably adequate for growth. Sedgwick (1979) observed that the rate of food consumption in the penaeid shrimp *Penaeus merguiensis* was related to the energy content of the diet, consumption being depressed at higher energy levels.

Growth of crayfish fed the high-protein (40%)–low-energy (2.5 kcal/g) diet 5 with a P:E ratio of 160 mg protein/kcal was markedly depressed compared to growth of crayfish receiving other low-energy diets. When the energy level of the 40% protein diet was increased to 3.5 kcal/g (diet 6) crayfish growth was significantly improved. Apparently there was insufficient nonprotein energy in the low-energy diet, thus protein was deaminated and utilized as an energy source. Though

TABLE 2.—Initial weights and lengths, percentage increases in weight and length, and percentage survival of crayfish fed experimental diets containing varying protein-to-energy (P:E) ratios. Values within a column without a letter in common are significantly different ($P \leq 0.05$).

Diet number	P:E ratio (protein and energy levels)[a]	Mean initial weight (g)	Mean initial length (mm)	Mean weight increase[b] (%)	Mean length increase[c] (%)	Survival (%)
1	80 (20, 2.5)	1.1	34.5	305.0 xyz	54.8 xy	100
2	57 (20, 3.5)	0.9	33.0	290.0 xyz	52.0 y	100
3	120 (30, 2.5)	1.0	32.7	369.2 x	64.6 x	94
4	86 (30, 3.5)	1.2	36.2	243.9 yz	37.6 z	88
5	160 (40, 2.5)	1.1	36.1	233.1 z	35.2 z	94
6	114 (40, 3.5)	1.0	34.8	330.5 xy	50.5 y	100

[a] Mg protein/kcal (% protein, kcal/g).

[b] Pooled SE for percentage weight increase was 13.6.

[c] Pooled SE for percentage length increase was 1.4.

more ammonia would have been produced as a result of deamination, water was continually circulated through each aquarium and it was assumed that this dilution effect would have negated adverse effects of the temporary rises in ammonia in individual aquaria. Dupree and Sneed (1967) and Prather and Lovell (1974) reported reduced growth in catfish fed high-protein diets containing inadequate nonprotein energy. As dietary energy was increased it apparently spared protein for growth. This sparing effect of carbohydrate and lipid has been demonstrated in finfish (NRC 1983), lobsters (Capuzzo and Lancaster 1979), and freshwater prawns (Clifford and Brick 1978, 1979).

Length increases of crayfish fed diet 3 were significantly greater than those of crayfish fed other diets except for those fed diet 1, which had a P:E ratio of 80 (Table 2). Length increases of crayfish fed diets with P:E ratios of 86 and 160 (diets 4 and 5) were depressed compared to those of crayfish fed other diets. These results are similar to the weight increase data and support the hypothesis that a dietary P:E imbalance affects growth of crayfish and perhaps is related to the amount of nonprotein dietary energy, which influences feed consumption and protein utilization.

Body composition data showed that crayfish fed diet 3 had significantly higher percentages of whole body protein than crayfish fed other experimental diets (Table 3). There was a significant increase in protein deposition as dietary energy decreased within each protein level. This suggests that the lower energy diets were adequate in energy and that at higher energy levels protein intake may have been restricted.

Significantly lower percentages of body fat were observed in crayfish fed low-energy diets than in those fed high-energy diets and fat. High levels of dietary energy or fat or both have been shown to increase body fat in various fish (Garling and Wilson 1976; NRC 1983). Body fat decreased significantly as dietary protein increased when crayfish were fed diets containing 3.5 kcal/g. Fat deposition in crayfish fed diets containing 2.5 kcal/g was not significantly different between crayfish fed 20 and 30% dietary protein, but did decrease significantly

TABLE 3.—Body composition (wet-weight basis) of juvenile red crayfish fed experimental diets containing varying protein-to-energy (P:E) ratios. Numbers within a column without a letter in common are significantly different ($P \leq 0.05$).

Diet number	P:E ratio (protein and energy levels)[a]	Dietary lipid (%)	Body composition (%)[b]			
			Moisture	Protein	Lipid	Ash
1	80 (20, 2.5)	2	78.65 xy	10.19 w	1.26 w	6.64 w
2	57 (20, 3.5)	10	78.47 xy	9.68 x	2.77 x	6.75 wx
3	120 (30, 2.5)	2	76.08 z	11.96 y	1.35 w	7.40 x
4	86 (30, 3.5)	10	77.67 y	11.00 z	2.28 y	5.99 w y
5	160 (40, 2.5)	2	79.64 x	11.24 z	0.81 z	5.95 y
6	114 (40, 3.5)	10	79.38 x	10.17 w	1.27 w	6.47 w y

[a] Mg protein/kcal (% protein, kcal/g).

[b] Pooled SEs were 0.16, 0.04, 0.05, and 0.07 for moisture, lipid, protein, and ash, respectively.

in those fed 40% dietary protein. These data may reflect increases in nonprotein energy requirements as dietary protein increases.

There were some significant differences in whole body ash percentages (Table 3). Crayfish fed diet 3 had significantly higher ash and lower moisture content than those fed other diets except for the ash content of crayfish fed diet 2. The significance of the observed differences in ash and moisture content is not known. There was no apparent trend that related ash and moisture content to dietary treatment.

Based on growth data for both length and weight, the optimum P:E ratio was between 80 and 120. Growth was not significantly different among crayfish fed diets 1 (P:E ratio of 80), 3 (P:E ratio of 120) and 6 (P:E ratio of 114). There were no improvements in growth of crayfish fed diets with higher or lower P:E ratios. However, based on body composition, a P:E ratio of 120 was optimum, since crayfish fed this diet exhibited higher levels of body protein. A P:E ratio of 120 is in the range (104–128) of optimum P:E ratios reported for certain other aquatic animals (NRC 1983).

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Intensive Feeding of Grass Carp in Ponds

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Abstract.—Growth and feed consumption were determined for yearling grass carp (*Ctenopharyngodon idella*) stocked at a rate of 8,000/hectare in earthen ponds and fed either a practical-type catfish diet or diets in which dehydrated alfalfa meal comprised either 19 or 38% of the formula. The diets were formulated to be approximately equal in estimated digestible energy (2.9 kcal/g) and digestible protein (27.7–28.4%) and to meet all nutritional requirements for channel catfish (*Ictalurus punctatus*). The fish grew from an average stocking size of 63 g to an average harvest size of 192 g with an average feed conversion of 2 g feed per gram of weight gain in 84 d. Fish fed the diet containing 19% alfalfa meal had faster weight gain and lower feed conversion ($P < 0.05$) than fish fed the other two diets. Analyses of digestive tract contents, before and after feeding, showed that natural pond foods accounted for only about 5% of the ingesta, by volume, indicating that supplemental feed provided most of the nutrients for fish growth. This study indicates that grass carp will grow rapidly beyond fingerling size in earthen ponds when fed concentrated supplemental feeds.

Commercial production of grass carp (*Ctenopharyngodon idella*) for aquatic weed control is actively practiced in several states in the United States. An important objective of commercial grass carp producers is growing the fish from fingerling to juvenile (30 cm total length) size as rapidly as possible because small fish are vulnerable to predation (Shireman et al. 1978a). A present practice for growing the fingerlings to juvenile size is to stock them in predator-free ponds in which macrophytes have been allowed to grow, but this does not allow for rapid growth under heavy stocking rates. Shireman et al. (1978b) fed catfish feed (32% protein) to grass carp but reported a poor growth response. Shelton et al. (1981) also fed a catfish feed (35% protein) to grass carp in ponds and reported similar results. They suggested that the poor growth could have been due to poor acceptance or poor digestibility of the supplemental diets by the grass carp. Shireman et al. (1978b) found that inclusion of a high quality roughage, such as duckweed (*Lemna* sp.) or alfalfa meal (*Medicago sativa*), in supplemental diets for grass carp improved growth rate of the fish. Although grass carp readily accept feeds containing high quality roughages, it is necessary that they consume diets with high nutrient contents in order to achieve a rapid rate of growth (Dabrowski 1977; Dabrowski and Kozak 1979).

The present study was designed to evaluate growth and feed acceptance by grass carp grown from fingerling to juvenile size in ponds and fed a practical-type catfish diet or a diet consisting of 19 or 38% dehydrated alfalfa meal. Alfalfa meal, made from the dried alfalfa plant, is commonly used in commercial diets for monogastric and ruminant animals and, among roughages, it is high in nutritional value and palatability.

Methods

Nine 0.1-hectare earthen ponds were each stocked with 800 age-0 grass carp averaging 12.5 g (10.2 cm) in late March. The fish were maintained through early June by offering, at a rate of approximately 2.5% of their body weight per day, a commercial catfish fingerling diet (0.2–0.25-cm-diameter crumbles) containing 36% crude protein.

Test feeding started on 8 June when the fish were large enough (58 g) to consume the pelleted experimental diets. Three ponds were randomly assigned to each of three dietary treatments: the catfish diet; the low-alfalfa diet; and the high-alfalfa diet (Table 1). The alfalfa diets were formulated to be equal to the catfish diet in estimated digestible energy and digestible protein, based on the crude protein and gross energy digestion coefficients for these ingredients that had been determined for another herbivore, blue tilapia (*Tilapia aurea*; Popma 1982). All diets were supplemented with vitamins and minerals to meet all nutritional requirements of channel catfish (*Ictalurus punctatus*).

The catfish diet was originally extruded into 0.8

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cm diameter particles by a commercial feed mill according to the prescribed formula. The particles were reground through a 0.24-cm screen in a hammer mill and repelleted with a laboratory model pellet mill (Astropelleter, London) into smaller size (0.48-cm-diameter) pellets for the grass carp. The ingredients for each of the two alfalfa diets were mixed, reground through the hammer mill, and pelleted in a similar manner. Soybean oil, which was used as a concentrated energy source, was added to the diets after pelleting to allow for a stronger bonded pellet. The oil was sprayed on with a hand sprayer as the dried pellets were rotated in a 25-kg-capacity mixer.

Based on observations during the maintenance period, the fish were fed the test diets at the rate of 2% of mean body weight per day. The feeding schedule was adjusted at 14-d intervals during the test period based on the body weight of sampled fish. Dissolved oxygen and water temperature were measured daily at the time of feeding. The feeding trial terminated on 31 August, 84 d after initiation.

On days 50 and 51 and again on days 74 and 75 of the feeding trial, fish were sampled to determine how well they were consuming the experimental diets. Five fish were sampled from each pond immediately before feeding and five more approximately 1 h after feeding for examination of digestive tract contents. The before and after collections were made on separate days to minimize the disturbance of feeding activity. As the fish were collected, they were covered with ice for rapid cooling to minimize digestion. After approximately 1 h they were placed in a storage freezer (−24°C) until examined.

For evaluation of food consumed, an area of the digestive tract located between the esophagus and the second loop, or about the anterior one-fourth of the digestive tract, was excised while the fish were partially frozen. Evaluation was restricted to this area because food particles distal to it were too digested for measurement or identification. In fish collected after feeding, most of the ingesta appeared to be in this anterior area of the digestive tract. Samples of the contents were examined under light microscope for identification of supplemental and natural food particles. The percentage by volume of supplemental and natural food in the samples was estimated visually by identifying and measuring individual particles in fields in a Sedgwick-Rafter cell as described by Devaraj (1976). After identifications were made, the total contents were removed from the excised section of the gut, dried (70°C for 24 h), and weighed.

TABLE 1.—Ingredient and estimated nutrient composition of three experimental diets fed to grass carp in ponds.

Measure	Catfish diet	Low-alfalfa diet	High-alfalfa diet
Ingredient (%)			
Alfalfa meal		19.3	38.5
Fishmeal	8	10.4	40
Soybean meal	56.5	49.4	14.6
Ground corn	31.7	13.6	
Dicalcium phosphate	1.5	1.3	1
Hemicellulose pellet binder	2.18	2.4	1.5
Vitamin premix[a]	0.1	0.1	0.1
Mineral premix[b]	0.02	0.02	0.02
Soybean oil		3.5	4.3
Digestible nutrient composition[c]			
Energy (kcal/g)	2.9	2.9	2.9
Protein (%)	28.1	27.7	28.4
Phosphorus (%)	0.52	0.54	0.52

[a] Vitamin premix provided the following per kilogram of feed: vitamin A, 4,000 international units (IU); vitamin D_3, 2,000 IU; niacin, 88 mg; d-pantothenic acid, 35 mg; vitamin B_{12}, 0.009 mg; vitamin E, 55 IU; riboflavin, 13 mg; choline chloride, 550 mg; menadione sodium bisulfate complex, 10 mg; pyridoxine hydrochloride, 10 mg; thiamine mononitrate, 10 mg; folic acid, 2 mg; ethoxyquin (antioxidant), 138 mg; ascorbic acid, 178.5 mg.

[b] Trace mineral premix provided the following per kilogram of feed: Mn, 86.5 mg; I, 1 mg; Cu, 4 mg; Zn, 150 mg; Fe, 33 mg; Co, 0.7 mg.

[c] Estimated from digestibility coefficients for the feedstuffs determined by Popma (1982) for blue tilapia.

Results and Discussion

Average growth rates of the grass carp during the preliminary feeding period and during the test period are shown in Figure 1. During the preliminary period 10 April to 7 June, when average weekly temperature ranged from 22.5 to 25.3°C, the fish grew from 12.5 to 58 g. The fish quickly adapted to the feeding area and consumed the feed vigorously.

The growth rate increased after the fish were changed from the acclimation crumble diet to the experimental pelleted diets. This increase could have been caused by increased water temperature, which caused the fish to consume more feed, or increased particle size of the diets, which minimized wasted feed; or both. The water temperature averaged 25°C during 24–31 May, increased to 33°C by 7 June when test feeding started, and remained above 32°C for most of the feeding period.

During the experimental period, average weight gain for fish fed the low-alfalfa diet was higher ($P < 0.05$) than it was for fish fed the catfish diet or the high-alfalfa diet (Table 2). Feed conversion

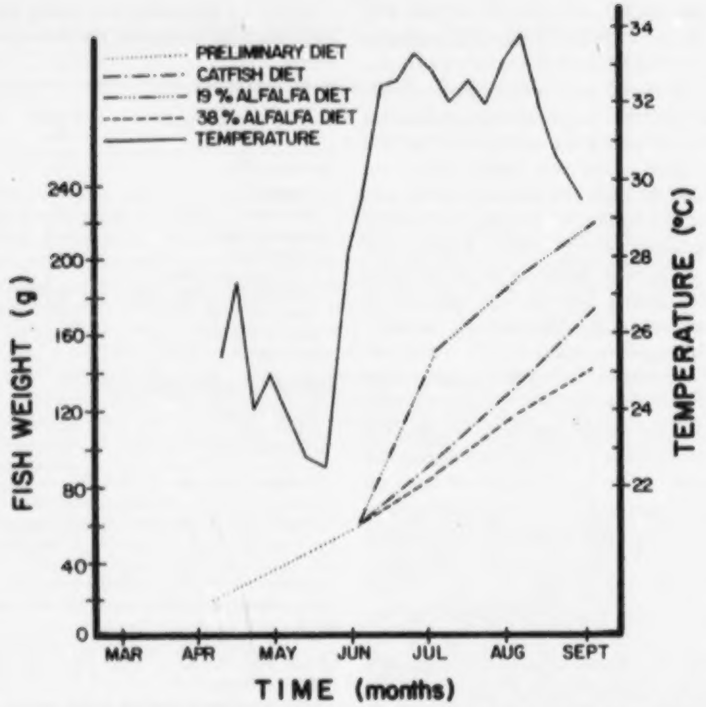

FIGURE 1.—Water temperature changes and growth responses of grass carp during preliminary feeding and during experimental feeding of a catfish diet, a 19% alfalfa diet, or a 38% alfalfa diet in 0.1-hectare ponds over a 6-month period.

for fish fed the low-alfalfa diet was lower ($P < 0.05$) than for fish fed the catfish diet or the high-alfalfa diet. These feed conversions were all lower than the 2.7 value reported by Shireman et al. (1978b) for grass carp fed catfish feed at a temperature of 25°C.

Fish collected from all treatment ponds 1 h after feeding had large amounts of ingesta in the examined area of their digestive tracts (Table 3). The lower part of the tract did not contain desirable digestible materials. The ingesta recovered after feeding contained about 95% supplemental feed. The weight of ingesta collected immediately prior to feeding, which consisted of natural food organisms and detrital materials, amounted to approximately 5% of that collected immediately after feeding.

TABLE 2.—Average harvest weight, weight gain per fish, and feed conversion for grass carp fed three formulated experimental diets in earthen ponds for 84 d. Means with similar letters in the same column are not different at $P < 0.05$.

Diet	Initial weight (g)	Harvest weight (g±SD)	Weight gain (g±SD)	Feed conversion[a]
Catfish	58	139±17 a	81±13 a	2.3 a
Low-alfalfa	61	216±18 b	155±14 b	1.4 b
High-alfalfa	56	166±15 a	110±12 a	2.2 a

[a] Grams of feed fed per gram of weight gain.

The relatively small quantity of natural food in the examined area of the digestive tracts implies that either natural food was relatively scarce under these culture conditions or feeding activity of the fish on natural food was reduced by the supplemental feeding. Ostracods, cladocerans, and *Pithophora* sp. were the most common natural foods seen in the intestine of the grass carp. Because the examination was limited to only one-fourth of the digestive tube, the relatively small amount of natural food found in the digestive tract at the time the analyses were made does not represent all natural food consumed, but indicates that grass carp depended primarily on the supplemental diets

TABLE 3.—Average contents (grams of dry matter) of the upper one-fourth of the digestive tracts of grass carp collected from ponds before feeding and 1 h after feeding, percentage (by volume) of the contents represented by natural foods, and percentage of the offered feed recovered from the digestive tract 1 h after feeding. Values for all criteria are not different ($P > 0.05$) among diets.

Diet	Digestive tract contents (g±SD)		Percentage natural food in contents (±SD)	Percentage of offered feed recovered
	Before feeding	After feeding		
Catfish	0.05±0.00	0.93±0.18	4.6±0.03	34
Low-alfalfa	0.13±0.04	1.44±0.11	7.6±0.02	55
High-alfalfa	0.04±0.01	1.24±0.23	3.2±0.02	35
Mean	0.07	1.20	5.1	41

rather than natural food for their growth. It was observed that grass carp as large as 28.2 cm consumed ostracods and cladocerans from the ponds. This agrees with the contention of Inaba and Nomura (1956) that grass carp are not obligate herbivores.

There were no differences ($P > 0.05$) in the total amount of ingesta or the amount of supplemental feed recovered from the digestive tracts of fish fed the various diets. The similarity in the amount of supplemental feed consumed by the fish in each treatment indicated that alfalfa meal did not significantly improve the acceptance of the low- or high-alfalfa diets over that of the catfish diet. An average 41% of the feed offered was recovered from the digestive tracts for all treatments. Failure to recover higher percentages of the feed offered may indicate that yearling grass carp will not eat more than approximately 1% of body weight at one feeding. Perhaps multiple feedings daily would increase consumption. The results, however, clearly indicate that grass carp consume supplemental feeds readily.

The modification of a catfish diet formula by the addition of 19% alfalfa meal improved growth rate of grass carp but did not increase acceptance of the diet. The reason for this improvement in growth rate is not known but it could be due to improved digestion resulting from the addition of the lower level of alfalfa to the diet whereas the higher level of alfalfa reduced digestion. The results of this study are in disagreement with those of other studies, which indicated that grass carp will not grow rapidly beyond fingerling size in earthen ponds when fed formulated supplemental feeds.

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Effect of Incubation Density and Substrate on the Development of Chum Salmon Eggs and Alevins

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Abstract.—Eggs from four families of chum salmon (*Oncorhynchus keta*) were incubated without gravel substrate at low and high densities until hatching. The alevins then were incubated at high or low density with or without substrate until emergence. Embryo survival rates were significantly different among families but not between incubation densities. Alevins incubated at low density with substrate had higher survival rates than those at low density without substrate and better survival than both high-density groups. Yolk-sac abnormalities and coagulated yolk in alevins and fry were least frequent among chum salmon incubated at low density with substrate; the alevins at low density had greater tissue weights at hatching than those incubated at high density. Fry emerging from low-density incubation with substrate were longer and heavier than those in all other groups. Egg and alevin densities and substrate quantity can be manipulated in standard hatching trays to enhance the survival, size, and quality of chum salmon fry.

Size of emergent salmon fry incubated under artificial conditions may be influenced by light (Disler 1953; Brannon 1965), water velocity and oxygen levels (Shumway et al. 1964), substrate (Marr 1963), and temperature (Heming 1982). Bams (1967) determined that standard hatchery incubation methods produced smaller fry than natural incubation conditions and that fry size at time of release was important to adult returns (Bams 1972). Alevin behavior may have changed in the presence of light, increased water velocity or temperature, or decreased oxygen levels or in the absence of a rugose substrate. These conditions increase alevin movements, depleting yolk reserves that would normally be used for growth (Bams 1969).

Artificially incubated fry may also develop yolk-sac abnormalities (Dumas 1966; Jochimsen and Bedell 1968). The yolk sac can become constricted at the distal end (Disler 1953) and in severe cases can rupture, causing the immediate death of the affected alevin (Fish and Burrows 1939). There can also be white, caseous, or coagulated yolk material in the yolk sac. This disorder has been described as coagulated yolk or white-spot disease (Mazuranich and Nielson 1959; Wolf 1970). Presence of substrate during incubation can reduce these problems (Banks 1983), but the relationship between substrate and elongated yolk sac or coagulated yolk has not been quantified. In our study, we examined the effects of egg density and the presence or absence of substrate during incubation on survival, rate of development, yolk-sac abnormalities, and alevin and fry size for individual families of chum salmon (*Oncorhynchus keta*).

Methods

Gametes were collected on January 16, 1984, from each of four pairs of chum salmon from the Chemainus River (48°51′54″N, 123°40′57″W) on southeastern Vancouver Island, British Columbia. In the laboratory, the eggs from each female were fertilized at 10.0°C with milt from a single male and the embryos were designated a family. Eggs were incubated at 10.0°C in two trays of a vertical stack incubator; each tray was partitioned into 20 compartments (7.0 × 7.5 × 5.0 cm) with a removable plastic divider. After 2 h water hardening, the eggs from each family were divided into 12 equal lots, six lots for each tray. Four compartments in each tray were assigned to each family, two for low egg density and two for high density. The low-density compartments each received one egg lot (average density 0.56 eggs/cm^3) while the high-density compartments each received two egg lots (0.97 eggs/cm^3). The trays were kept in darkness, and were provided with dechlorinated tapwater at a flow of 7.0 L/min. Oxygen concentrations were above 90% of air saturation and densities averaged about 3,200 eggs/tray. These conditions were within acceptable limits for normal salmonid development (Bams and Simpson 1977). Water temperatures were recorded daily and the mean temperature from fertilization to emergence was 10.0 ± 0.18°C (SD).

The incubation trays were checked daily and dead eggs were removed from each compartment and stored in Stockard's solution (Rugh 1952), except during the critical stage of epiboly when the embryos would be extremely sensitive to movement (Smirnov 1975; Johnson et al. 1983). All dead eggs were inspected to determine if they were fertilized. Eggs which showed no sign of cellular development were considered unfertilized and were excluded from the calculation of survival rates.

During hatching, alevins were counted daily. Hatching date was considered as the time when 50% of the alevins hatched. Within 1 d of this date, we removed 10 alevins from each compartment and preserved them in 10% formalin. After all the alevins had hatched, a low- and a high-density group of alevins from each family were removed from each tray and gravel (2.0–3.0 cm in diameter) was placed in the bottom of the compartment. The alevins were then returned to their appropriate compartments, so that each family had a low- and a high-density group with and without substrate in each tray. The addition of substrate increased the average density in the low-density compartments from 0.53 to 0.68 alevins/cm^3 (we assumed an 80% void space within the substrate) and in the high-density compartments from 0.90 to 1.18 alevins/cm^3.

Lack of visible yolk is one criterion used to distinguish alevins from fry and is associated with emergence from the gravel (Bams 1969; Mason 1976). When 50% of the alevins had become fry, all of the alevins and fry in a compartment were preserved in 10% formalin.

All samples were preserved for at least 90 d to ensure that increases in weight and decreases in length had ceased (Heming and Preston 1981). Fork lengths of alevins and fry were measured to the nearest 0.1 mm with Vernier calipers. Total weights were determined by blotting the individuals dry and weighing them to the nearest 1.0 mg. Yolk was separated from the body tissue of alevins and fry and weighed. Tissue weight was determined by subtracting yolk weight from total weight.

Body shape was determined for 50 live alevins from each compartment at 50% yolk absorption (stage 32: Vernier 1969). Body shape was classified, from the illustrations in Dumas (1966), Jochimsen and Bedell (1968), and Emadi (1973), into three categories: (1) no elongation of the distal end of the yolk sac; (2) partial elongation of the distal end of the yolk sac; and (3) severe elongation with constriction of the yolk sac and the presence of coagulated yolk. After examination, the live alevins were returned to their appropriate compartments. Because the presence of coagulated yolk reduces the survival of fry during the early stages of feeding (Banks 1983), we examined 50 fry from each of the preserved fry samples for the presence of coagulated yolk.

Differences in survival rate, body shape, and presence of coagulated yolk between incubation densities, between present and absent substrate, and among families were tested by the likelihood ratio statistic (*G*-test: Sokal and Rohlf 1981). An approximate *F*-ratio (*G*-statistic ÷ df) was used to test the relative magnitude of the source of variation. The 50% hatching times were calculated by the method of Colby and Brooke (1969). Equality of alevin length and weight between densities was tested by analysis of variance. Equality of fry length and weight among densities and substrates was tested with the analysis of variance model

$$Y_{ijk} = \mu + D_i + S_j + DS_{ij} + e_{ijk};$$

Y = length or weight;
μ = mean;
D = density (i = 1, 2);
S = substrate (j = 1, 2);
DS = density and substrate interaction;
e = residual error.

Results

Survival Rates

Survival rates of Chemainus River chum salmon eggs, incubated at 10°C and at different densities from fertilization to hatching, were greater than 94% for all treatments (Table 1). There were significant differences in survival rates among families (Table 2), two families having significantly lower survival rates (92.8 and 94.5%) than the other two families (97.0 and 97.2%). Differences in embryo survival rates were greater among families than between incubation densities; differences between densities were not significant (F = 8.03; df = 12, 2; $P > 0.05$). Embryos incubated at low or high density survived equally well.

The addition of substrate enhanced alevin survival at low alevin density, but depressed it at high density (Table 1). Differences in alevin survival rates were greater between incubation densities than between substrate treatments; and both these differences were significantly greater than those among families or between replicates (Table 2). Most alevin mortality occurred after 50% yolk absorption and those alevins that died showed evidence of severe elongation of the yolk sac and coagulated yolk.

TABLE 1.—Percent survival and density of embryos and alevins for Chemainus River chum salmon incubated at 10°C. Families are pooled for each treatment.

Incubation density and substrate	Percent survival (%)			Density (SD)	
	Fertilization to hatching	Hatching to emergence	Fertilization to emergence	Eggs·cm²	Alevins·cm²
Low density, no substrate	95.4 (1.164)	99.3 (1.110)	94.7	0.56 (0.08)	0.53 (0.07)
Low density, substrate	95.6 (1.793)[a]	95.7 (1.341)	91.5	0.56 (0.08)	0.68 (0.09)
High density, no substrate	95.7 (1.576)	89.1 (1.891)	85.3	0.97 (0.05)	0.90 (0.06)
High density, substrate	94.8 (2.087)[a]	83.7 (1.977)	79.4	0.97 (0.06)	1.18 (0.10)

[a] No substrate was present from fertilization to hatching.

While monitoring alevin survival between hatching and fry emergence, we observed that there were behavioral differences among alevins incubated at different densities with and without substrate. Alevins incubated at high density with and without substrate were very active and tended to crowd together in a vertical position with their heads down. This behavior caused the oil globule in the yolk to migrate to the distal end of the yolk sac and, as development continued, caused the elongation of the yolk sac. All alevins with severe yolk-sac elongation also had coagulated yolk. Alevins at low density without substrate were also very active and tended to crowd together but they remained in a horizontal position; those incubated with substrate were less active and also tended to remain in a horizontal position.

Body Condition

We examined the variation in body shape of alevins at 50% yolk absorption (Table 3). There were striking differences in the frequencies of body-shape type associated with incubation density and presence or absence of substrate. The addition of substrate at low alevin density reduced yolk-sac malformation but increased it at high density. Most alevins (73%) at high density with substrate had severe elongation of the yolk sac and coagulated yolk. At both low and high density without substrate, the incidence of severe elongation and coagulated yolk was low (1–3%), but fewer alevins showed elongation of the yolk sac at low density (54%) than at high density (81%). There were significant differences in body shape among the four

TABLE 2.—Likelihood ratio (*G*-tests) of differences in survival rates of Chemainus River chum salmon embryos and alevins between densities, substrates, and replicates and among families. Asterisks denote significant *G*-values at $P < 0.05$*.

Comparison	df	Fertilization to hatching[a]		Hatching to emergence		Fertilization to emergence	
		G	Standardized statistic	*G*	Standardized statistic	*G*	Standardized statistic
Between densities	2	1.07	0.53	113.01*	56.50	89.54*	44.77
No substrate	1	0.12		0.47		0.74	
Substrate	1	0.95		112.54*		88.80*	
Between substrates	2	1.59	0.79	67.01*	33.50	54.69*	27.34
Low density	1	0.05		42.57*		29.52*	
High density	1	1.54		24.44*		25.17*	
Among families	12	51.08*	4.26	50.97*	4.25	80.70*	6.72
Low density, no substrate	3	11.98*		24.56*		34.97*	
Low density, substrate	3	10.70*		2.19		7.73	
High density, no substrate	3	14.58*		10.97*		16.24*	
High density, substrate	3	13.82*		13.25*		21.76*	
Between replicates	4	0.12	0.03	2.29	0.57	1.73	0.43
Low density, no substrate	1	0.01		0.23		0.30	
Low density, substrate	1	0.09		0.22		0.44	
High density, no substrate	1	0.01		1.83		0.99	
High density, substrate	1	0.01		0.01		0.00	
Total	20	53.86		233.28		226.66	

[a] Substrate was not present from fertilization to hatching.

TABLE 3.—The frequency of different alevin body shapes at 50% yolk absorption and frequency of coagulated yolk in chum salmon fry with no visible yolk. *N* is the number of live alevins or preserved fry in each treatment (replicates combined) of which 50 alevins or fry were examined per replicate. Body shape designations are (1) no elongation, (2) partial elongation and no coagulated yolk, and (3) severe elongation of yolk sac and presence of coagulated yolk. Gut condition was recorded as (1) no coagulated yolk and (2) coagulated yolk.

	No substrate								Substrate							
	Low density				High density				Low density				High density			
Family	*N*	1	2	3	*N*	1	2	3	*N*	1	2	3	*N*	1	2	3
								Alevin body shape								
1	275	0.36	0.62	0.02	452	0.13	0.85	0.02	299	0.79	0.21	0.00	501	0.03	0.33	0.64
2	285	0.64	0.36	0.00	472	0.29	0.71	0.00	291	0.98	0.02	0.00	502	0.00	0.58	0.42
3	268	0.44	0.56	0.00	511	0.09	0.91	0.00	306	0.76	0.24	0.00	493	0.00	0.00	1.00
4	282	0.40	0.57	0.03	456	0.25	0.65	0.10	245	0.86	0.14	0.00	481	0.00	0.13	0.87
Total	1,110	0.46	0.53	0.01	1,891	0.19	0.78	0.03	1,141	0.84	0.16	0.00	1,977	0.01	0.26	0.73
								Fry gut condition								
1	262	0.55	0.45		416	0.21	0.79		290	1.00	0.00		439	0.81	0.19	
2	236	0.64	0.36		414	0.36	0.64		279	1.00	0.00		408	0.77	0.23	
3	238	0.63	0.37		439	0.46	0.54		291	1.00	0.00		421	0.82	0.18	
4	244	0.66	0.34		414	0.54	0.46		232	1.00	0.00		387	0.72	0.28	
Total	980	0.62	0.38		1,683	0.39	0.61		1,092	1.00	0.00		1,655	0.79	0.21	

families in all treatments. For example, at high density with substrate, all family-3 alevins but only 42% of family-2 alevins had severe elongation of the yolk sac and coagulated yolk. The significant differences between replicates may have resulted from slight differences in alevin density between replicates and from the nonuniform nature of the gravel used as substrate. Variability in body shape was substantially greater between density and substrate treatments than between replicates or among families (Table 4).

The addition of substrate reduced the frequency of yolk-sac elongation at low incubation density, but greatly increased it at high density, probably by raising the already high alevin density from 0.90 to 1.18 alevins/cm^3. Alevins incubated at low and high densities without substrate had intermediate frequencies of misshapen bodies, low-density alevins being the less affected.

The presence or absence of a substrate and the incubation density had a marked effect on the occurrence of coagulated yolk in the guts of fry. Fry incubated at low density with a substrate had no coagulated yolk in their guts, whereas 61% of the fry incubated at high density without substrate had coagulated yolk (Table 3). Overall, substrate had the greatest effect on the frequency of coagulated yolk in fry than any other treatment variable (Table 4). Low alevin density also reduced the frequency of coagulated yolk in fry. Differences in the frequency of coagulated yolk were substantially greater between densities and substrates than among families or between replicates.

Hatching and Emergence Time

Hatching time of alevins and emergence time of fry were similar for all families and all treatments. At 10°C, 50% of alevins hatched 51.9 ± 0.48 d (mean ± SD) after fertilization, and 50% of alevins developed into fry 89.1 ± 0.45 d after fertilization. Incubation conditions did not affect the rate of development of chum salmon embryos and alevins.

Alevin and Fry Size

Alevins hatched at low and high density without substrate had similar fork lengths, total weights, and yolk weights (Table 5). Alevins hatched at low density had greater tissue weights than those hatched at high density ($F = 8.74$; df = 1, 318; $P < 0.05$). Those alevins continuing development with or without substrate at either density were similar in size. Fry that developed at low density were longer ($F = 6.15$; df = 1, 144; $P < 0.05$) and had greater wet weight ($F = 5.98$; $P < 0.05$), lower yolk weight ($F = 10.55$; $P < 0.05$), and greater tissue weight ($F = 12.98$; $P < 0.05$) than those developing at high density. The effect of substrate depended upon alevin density. At low density, alevins provided with substrate developed into fry that were longer and heavier than those incubated at low density without a substrate (Table 5). At high density, however, alevins provided with a substrate produced fry that were of similar length and weight to those incubated without a substrate. These results suggest that fry produced at low den-

TABLE 4.—Likelihood ratio (G-test) of differences in body shapes of alevins at 50% yolk absorption and of coagulated yolk of fry with no visible yolk between densities, substrates, and replicates, and among families, of Chemainus River chum salmon. Asterisks denote significant G-values at $P < 0.05$*.

	Alevin			Fry		
Comparison	df	G	Standardized statistic	df	G	Standardized statistic
Between densities	4	912.30*	228.08	2	123.54*	61.77
No substrate	2	59.09*		1	21.92*	
Substrate	2	852.71*		1	101.62*	
Between substrates	4	653.01*	163.25	2	297.78*	148.89
Low density	2	150.75*		1	177.97*	
High density	2	502.26*		1	119.81*	
Among families	24	223.80*	9.32	12	18.60*	1.55
Low density, no substrate	6	18.77*		3	1.81	
Low density, substrate	6	28.01*		3	0.00	
High density, no substrate	6	45.19*		3	12.83*	
High density, substrate	6	131.83*		3	3.96	
Between replicates	8	85.19*	10.65	4	0.67	0.17
Low density, no substrate	2	40.23*		1	0.01	
Low density, substrate	2	0.02		1	0.00	
High density, no substrate	2	10.34*		1	0.02	
High density, substrate	2	34.60*		1	0.64	
Total	40	1,874.30		20	440.59	

sity with a substrate converted yolk to tissue more efficiently than fry produced in the other three treatments.

Discussion

Under natural spawning conditions, gravel provides the appropriate substrate and protection against light during incubation of salmonid eggs and alevins. Unlike alevins in nature, the alevins in hatchery trays lack a rugose substrate and lie on the bottom of the tray. However, the alevins retain their behavioral adaptations (Bams 1969) and when disturbed or stressed they become very active. To seek shelter, they cluster on top of each other and try to force their way downward. The downward orientation causes the oil globule in the yolk sac to shift posteriorly (Disler 1953). If the stress continues for a protracted time, the shift in the oil globule and continued absorption of the yolk result in elongation of the yolk sac. This often causes the oil globule and some yolk to be pinched off and yolk to coagulate; this, in turn, can affect subsequent growth and survival (Leon 1975).

Embryo survival rates of chum salmon from fertilization to hatching may range from 35 to 100% when eggs are incubated without substrate (Beacham and Murray 1986). Our study indicates that embryo survival rates differ among families but not between low (0.56 alevins/cm^3) and high (0.97/cm^3) incubation densities. Banks (1983) found that differences in the type of substrate (biorings, bio-saddles, or netting), the time it was provided (7 d before or 2 d after hatching), or its arrangement (random or systematic) had no apparent effect on the survival of chinook salmon fry (*Oncorhynchus tshawytscha*). Leon (1982) incubated chum salmon eggs at two densities (0.43 and 0.55 alevins/cm^3) and found that the high density reduced egg-to-fry survival. We found that chum salmon alevins incubated with substrate had higher survival rates at low density than at high density. Alevin survival rates without substrate did not differ from each other, but were higher than the survival rates of alevins incubated with substrate at high density. This suggests that the addition of substrate at high incubation density reduces survival in shallow matrix incubators, probably by increasing crowding and behavioral interactions of the alevins. However, Kepshire (1982) found that survival rates for five species of Pacific salmon cultured in deep matrix incubators with substrate were not a function of alevin density. With densities ranging from 0.12 to 2.83 alevins/cm^3, survival rates were greater than 90% for all species. Deep matrix incubators probably simulate natural conditions better, and allow more natural behavioral responses, than shallow matrix incubators.

Leon (1975) and Leon and Bonney (1979) noted that incubation of Atlantic salmon (*Salmo salar*) alevins without substrate produced a higher frequency of elongated and constricted yolk sacs than incubation without substrate. Emadi (1973) found

TABLE 5.—The effect of incubation density and presence of substrate on fork length and on total, yolk, and tissue wet weights of Chemainus River chum salmon alevins at 50% hatch and at 50% development to fry (no visible yolk). Values are means (SEs).

	Low density		High density	
	Substrate[a]	No substrate	Substrate[a]	No substrate
	Alevins (N = 80/treatment)			
Fork length (mm)	22.6 (0.08)	22.6 (0.07)	22.5 (0.07)	22.8 (0.07)
Total weight (mg)	257.9 (3.33)	261.9 (3.29)	252.3 (3.58)	258.4 (3.12)
Yolk weight (mg)	143.8 (1.70)	144.6 (1.64)	146.0 (1.95)	145.5 (1.59)
Tissue weight[b] (mg)	114.1 (2.03)	117.3 (1.93)	106.3 (2.20)	112.9 (2.04)
	Fry (N = 112/treatment)			
Fork length (mm)	34.7 (0.09)	34.5 (0.07)	34.1 (0.09)	34.5 (0.08)
Total weight (mg)	353.1 (2.86)	337.7 (2.70)	339.8 (3.15)	336.7 (2.49)
Yolk weight (mg)	23.7 (0.67)	23.6 (0.82)	27.5 (0.82)	24.8 (0.72)
Tissue weight[b] (mg)	329.4 (2.52)	314.1 (2.52)	312.3 (2.95)	311.9 (2.68)

[a] Substrate was not present from fertilization to hatching.

[b] Tissue weight = total weight − yolk weight.

that warm temperatures and high water velocities aggravated the incidence of elongated yolk sacs and coagulated yolk and that addition of substrate tended to reduce the condition. We also found that the addition of substrate resulted in a lower frequency of elongated yolk sacs when chum salmon were incubated at low density. Without substrate, however, the frequency of elongated yolk sacs was lower at high than at low density. This possibly occurred because at high density without substrate the alevins tended to prop each other up, forming a living substrate that reduced the incidence of elongated yolk sac. Banks (1983) found that, although chinook salmon fry losses were considerably lower when substrate was used, coagulated yolk was also evident in the fry. We also found that substrate did not totally eliminate either coagulated yolk or elongated yolk sacs and, at high density, the addition of substrate actually increased the frequency of malformed yolk sacs by increasing alevin density. Yolk-sac malformations were also variable among families, possibly because of familial differences in alevin behavior. These results suggest that the interactions among density, substrate, and behavior can play important roles in the occurrence of elongated yolk sacs and coagulated yolk in alevins and fry.

The incubation of salmonid alevins with substrate produced longer and heavier fry than those from systems utilizing no substrate (Bams 1967; Kepshire 1982; Leon 1982; Banks 1983). There is also evidence that increased egg-loading density in both shallow and deep matrix incubators tends to decrease the length and weight of sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), and chum salmon fry at emergence (McDonald 1977; Bailey et al. 1980; Bams 1982; Kapuscinski and Lannan 1983). Our results for chum salmon suggest that the addition of substrate produced larger fry when incubation density was low, but had little effect on the size of fry at high density.

Our findings and those of others suggest it is possible to enhance the survival and size of salmonid fry by including a substrate during incubation. We suggest that elongated yolk sacs and coagulated yolk result from the normal behavioral response of chum salmon alevins to disperse downward after hatching. Incubation in traditional hatchery baskets and trays prevents this migration. The addition of substrate in shallow incubation systems, particularly at low embryo density, allows more normal behavioral responses and results in greater survival and larger fry. However, as pointed out by Bams (1983), the criteria and circumstances used in determining fry quality are complex and must be examined along with the advantages and disadvantages of each incubation system before the most successful system can be determined.

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Arthur Demsky, Eric Lauinger, and Karen Shillington weighed and measured the chum salmon eggs, alevins, and fry examined in this study and also determined the stage of development of the eggs. Bruce Bernard assisted greatly in the data analysis. Alf Stefanson's assistance in the collection of the adult chum salmon from the Chemainus River is also greatly appreciated.

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Effects of Diet on Growth and Survival of Larval Walleyes

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Abstract.—The effects of diet quality on larval walleye (*Stizostedion vitreum vitreum*) growth and survival are described. The cyclopoid copepod *Diacyclops thomasi* consumed larval walleyes within 10 min at dense copepod concentrations and within 1 d at lower densities (500 organisms/L). At initial feeding, larval walleyes consumed both copepods and cladocerans 500–1,100 μm total length. Postlarva-II walleyes fed four different diets (minnow larvae, brine shrimp, and two size grades of zooplankton) for 6 d averaged 4.00 mg, 3.48 mg, 2.23 mg, and 1.92 mg (dry weight), respectively. No appreciable differences in survival (70–83%) were observed on these diets. During the first 3 weeks of life, the survival of walleye larvae at optimal conditions of diet, temperature, light, space, and density was 71% (range, 52–87%). The combined influence of higher water temperatures and smaller food organisms stimulated initial feeding 3 d sooner than previously reported for larval walleyes. Sufficient food and higher temperatures may be factors in suppression of cannibalism. *Artemia* sp. may be the best choice of a first food, because brine shrimp support high growth and survival of larval walleyes and do not prey on the larvae as do some copepods.

Pond culture of walleye (*Stizostedion vitreum vitreum*) larvae has been used exclusively in the artificial propagation of this species to insure adequate space and nutritional needs of larvae (Dobie 1956). Solution of environmental problems requires that environmental requirements of important indigenous biota be established, usually by laboratory testing. Attempts to culture walleye larvae in the laboratory have given mixed results. Mass culture techniques, involving high densities and numbers of larvae fed artificial food, were largely unsuccessful (Beyerle 1975; Nickum 1978). Survival of walleye larvae fed live zooplankton was not much better, varying from 0.4% in an 11-d period (Olson 1974) to 14.1% in 18 d (Raisanen and Applegate 1983). The intrinsic biological problem is that cannibalism among larvae is greatest during the first 20 d after hatching (Cuff 1977; Li and Mathias 1982).

Survival of larval walleyes can be enhanced by optimizing several experimental variables. Smith and Koenst (1975) observed that all larvae died at the four lowest temperatures studied (6.0–15.0°C); at 21°C, 8% of the larvae survived to the juvenile stage. Hokanson and Kleiner (1974) observed optimum yields (85% survival) of swim-up yellow perch larvae (*Perca flavescens*) when initial incubation temperatures were increased 0.5–1.0°C/d (range, 4.9–24.3°C). Hokanson and Koenst (1986) established 26°C as the optimum temperature for growth of juvenile walleyes. Larvae and juveniles are known to have similar thermal requirements, which suggests that larvae can be cultured at higher temperatures than are commonly used (<20°C). Considerable progress in walleye culture was made when Li and Mathias (1982) described the importance of food density, timing of food availability, and fish density and their relationships to rearing unit volumes. However, cannibalism was never under control and mortality was unacceptably high. Optimal density of larvae was found to be less than 1 larva/L, which was denser than obtained in pond culture, 0.02–0.4 larva/L (Cheshire and Steele 1972). Li and Mathias determined that a volume of 20 L was the minimum space needed by their larval walleyes. Olson (1974) found that containers with only 8.9 cm of water were unsuitable for rearing walleye larvae. Walleye larvae are positively phototactic during the first 8 weeks of life (Bulkowski and Meade 1983). Mathias and Li (1982) observed that zooplankton were eaten commonly during daylight in ponds, but their occurrence in stomachs of larval walleyes decreased at night and disappeared completely just before dawn; fish and gammarids were consumed more effectively during the night. The larger young of the year had three distinct feeding peaks: one before dusk, one after dusk, and one at midmorning. This information becomes important when one plans the frequency of feeding to suppress cannibalism.

The present study was designed to demonstrate the importance of food quality in the nutrition and health of walleye larvae. Specific objectives were to (1) describe the effects of predaceous zooplankton on walleye larvae, (2) describe the first food of walleye larvae in aquaria based on zooplankton size frequencies and taxa consumed, (3) describe the effects of four diets (larval fathead minnows *Pimephales promelas*, brine shrimp *Artemia salina* nauplii, and two size grades of zooplankton) upon the growth and survival of larval walleye, and (4) describe the results of culturing walleyes for the first 3 weeks of life under optimal conditions.

Methods

The project was divided into four discrete experiments (Table 1). Gametes were obtained in April 1983 for experiments 1–3 and in April 1984 for experiment 4. All embryos were raised on a rising temperature regimen (1°C/d) from 6.7 to 17.7°C for optimum survival (Hokanson and Kleiner 1974). The embryos were incubated in Lake Superior water with a mean flow of 190 mL/min (range, 75–220 mL/min). Dead and infertile ova were removed daily to control growth of *Saprolegnia* sp. Normal hatch for ova varied within 55–88% for the Lower Fox River walleye stock, 33–79% for the Little Cutfoot Sioux Lake stock, and 75–92% for the Chippewa Flowage stock. The pH during incubation was 7.8 (range, 7.5–7.9) and average dissolved oxygen during incubation was 94% of air saturation (range, 91–101%). All larvae were reared in static test systems (beakers and aquaria) with Lake Superior water; beakers were aerated initially and aquaria received continuous aeration. Dissolved oxygen was 91–101% of saturation; pH was near neutral (range, 7.8–8.4). Continuous day length was provided by Vita-lite fluorescent bulbs to encourage walleye larvae to feed on smaller plankton (Mathias and Li 1982) and to discourage cannibalism.

Live zooplankton were collected every other day from ponds at the Environmental Research Laboratory–Duluth (ERL–D) by hand-towed Wisconsin style plankton nets (80-µm-mesh screen). Field samples were passed through a 505-µm-mesh

TABLE 1.—Experimental conditions in larval walleye rearing trials.

Gamete source	Life phase[a]	Volume test unit	Number of larvae per unit	Diet	Food source[b]	Mean temperature, °C (range)
			Experiment 1: predaceous zooplankton			
Lower Fox River, Wisconsin	P_1	500 ml	4	*Zooplankton*[c] (16,000/L)	S	20.6
	P_1	500 ml	4	*Zooplankton*[d] (dense)	S	20.6
	P_1	500 ml	4	*Zooplankton*[d] (500/L)	S	20.6
	P_1	500 ml	4–10	*Zooplankton*[e]	L, S	20.6
			Experiment 2: first feeding			
Lower Fox River	P_2	20 L	100	Zooplankton[c] (500/L)	S	20.6
Little Cutfoot Sioux Lake, Minnesota	P_2	20 L	100	Zooplankton[c] (500/L)	S	20.6
			Experiment 3: diet quality			
Little Cutfoot Sioux Lake	P_3	20 L	10/diet	*Pimephales promelas*[f]		18.8 (18.4–19.3)
				Artemia salina[g]		
				Zooplankton[h] (200/L·d)	S	
			Experiment 4: optimal conditions			
Chippewa Flowage, Wisconsin	P_1–P_4	40 L	100 (P_1) 20 (P_4)	*Artemia salina*[i]		21.8 (21.3–22.5)

[a] P_1 = prolarva; P_2 = postlarva I; P_3 = postlarva II; P_4 = juvenile (Li and Mathias 1982).

[b] S, small pond containing *Diacyclops thomasi* and *Daphnia rosea*. L, Large pond with diverse assemblage including *Polyarthra* and copepod nauplii.

[c] Passed through a 505-µm-mesh screen.

[d] Retained by a 333-µm-mesh screen.

[e] Retained by 202-, 157-, and 110-µm-mesh screens from each of two ponds.

[f] Newly hatched; 6.04 mm total length; 0.109 mg dry weight.

[g] Great Salt Lake source (Biomarine); nauplii averaged 629 µm; 2 d at 18–20°C.

[h] Zooplankton retained by 202- and 333-µm-mesh screens.

[i] Strain from Colombia, South America (average 705 µm; 2 d at 28.0°C). Six days after hatch, larvae were fed artemia from the northern basin of Great Salt Lake (average 740 µm; 2 d at 28.0°C).

screen to remove debris and larger zooplankton and poured into a 40-L plastic garbage can. In the laboratory, the zooplankton stock volume was adjusted to 35 L with Lake Superior water and moderately aerated at room temperature. Before each daily feeding, the mean density of live zooplankton in the stock was determined. Three 2–10-mL subsamples were collected with a Hensen–Stempel pipette near the surface of the aerated volume and the number of live zooplankton was counted on a plankton-counting wheel mounted on a binocular dissecting scope to determine the volume of zooplankton stock to feed each aquarium. One food ration was randomly selected from each stock and preserved in buffered formalin for length-frequency measurements and taxonomic identification. Total lengths, less appendages, were measured to the nearest 50 μm with an ocular micrometer for artemia, copepods, and cladocera. Counts and taxonomic identification were performed on from two to five subsamples taken from a 200-mL flask with a 1-mL Stempel pipette. Each whole sample was examined for rare species. Copepoda and some cladocera were identified to species by taxonomic keys according to Ward and Whipple (1959) and *Daphnia* spp. were identified according to Brandlova et al. (1972).

Experiment 1: effects of predaceous zooplankton.—Several discrete tests were conducted to document the impact of predaceous zooplankton on walleye larvae during initial feeding trials (Table 1). In the fourth test, zooplankton from two pond sources, retained by 202-, 157-, and 110-μm-mesh screens, were used as food.

Experiment 2: first food of larvae.—First-feeding observations were conducted in 20-L aquaria on postlarva-I walleyes of 0.49–0.81 mg mean dry weight. Larvae from individual females (six females from Wisconsin, four from Minnesota) were reared in separate aquaria. Two 500-mL subsamples were taken from each zooplankton stock to characterize the size frequency and taxa offered as food. After larvae had fed for several hours, a subsample of 9–38 walleye larvae was taken from each aquarium and preserved in 4% buffered formalin; later, the gut contents were examined with a binocular dissecting scope. The total lengths (less appendages) of intact zooplankton consumed were measured to the nearest 50 μm with an ocular micrometer. The linear forage ratio (L) of Strauss (1979) was used to determine species and size selection of zooplankton by walleyes. The index is $L_i = r_i - p_i$, where r_i is the relative abundance of prey i in the stomach and p_i is the relative abundance of prey i in food samples. Values of L range from +100 to −100%; positive values indicate selection for food types and negative values indicate avoidance of them.

Evidence for food selection was examined by testing the hypothesis that the proportions of food offered and eaten were the same for all prey-size categories. The size categories with low numbers of prey were combined to avoid problems with zero counts in some categories. The resulting data (prey counts by size category expressed as a percentage of the total number of prey in the sample), were subjected to arcsine transformations (Dixon and Massey 1969). The data were analyzed by weighted multivariate one-way analysis of variance with SAS statistical programs (SAS Institute, Cary, North Carolina). The weights were the total number of prey in a sample. Linear dependencies in the proportions occurred; therefore, the data for one size category could not be used. To avoid the arbitrary selection of which measure to eliminate, the significance probability of the geometric mean F from all possible analyses with one parameter omitted is reported.

Experiment 3: diet quality.—Walleyes at the postlarva-II phase of development were selected for the diet study; walleyes at this phase of development are highly cannibalistic. The four diets fed were larval fathead minnows, artemia nauplii, and two size grades of zooplankton (Table 1). Larvae from two females provided duplication.

Nauplii were fed to walleye larvae three times daily at 0700, 1500, and 2300 hours. Excess rations of larval fathead minnows and artemia were provided. Zooplankton were fed to larvae at a rate of 200 organisms/L daily. The volume of zooplankton stock solution (V, liters) added to each aquarium was determined by the formula:

$$V = \frac{\text{nominal rations (200 organisms/L)} \times 19\ \text{L}}{\text{mean live count (number/L)}}.$$

The desired volume of zooplankton stock solution was determined daily for each size fraction and measured to the nearest 10 mL. Black plastic surrounded each aquarium to assist larval perception of prey (Hale and Carlson 1972); light intensity averaged 93 lx (range, 59–135 lx).

The measured responses were percent survival and dry weight of individual larvae, the latter obtained by oven-drying specimens to a constant weight at 60°C. Daily specific rates of growth, mortality, and net biomass change were derived from the procedures of Hokanson et al. (1973). The diet study was conducted for 6 d. Treatment differences were determined from a randomized block design with Duncan's new multiple-range test ($P = 0.05$).

Experiment 4: optimal culture conditions.— Walleye larvae were cultured through the cannibalistic stage under optimal conditions established in several previous studies (Table 1). Eggs were obtained from six females. Experimental conditions for larva culture were similar to those of experiment 3; light intensity averaged 1,485 lx (range, 1,184–1,830 lx). Artemia were fed to walleye larvae at 8-h intervals daily (0630, 1430, and 2230 hours) to encourage continuous feeding. After the walleye larvae had resorbed the oil globule, this diet was supplemented with a surplus number of 0–5-d-old fathead minnow larvae (5.0–8.4 mm total length) or yellow perch larvae (4.7–6.6 mm total length). The dead artemia and wastes were siphoned daily. Observations on walleye survival and growth were made at discrete intervals of ontogeny (Li and Mathias 1982) for the first 3 weeks of life.

Results and Discussion

Effects of Predaceous Zooplankton

Several tests were performed that document the effects of zooplankton upon survival of walleye larvae. The larvae placed into the dense concentration of zooplankton, offered as food, disappeared within 10 min in the first test, presumably by predation. In the second test, the walleye larvae placed into the strained concentration were consumed by the zooplankton within 15 min. A third test was made at a more nominal density of zooplankton (500 organisms/L) used for feeding larvae (Li and Mathias 1982). All walleye larvae disappeared over a 12-h period, presumably consumed by zooplankton. The zooplankton stock contained mostly copepod juveniles retained by a 333-μm-mesh net. Therefore, another source of zooplankton was used for feeding trials in the fourth test. All walleye larvae remained alive after about 24 h in media containing zooplankton that were retained by 110- and 157-μm-mesh nets from both large and small ponds and by 202-μm-mesh nets from the large pond. Only 25% of larvae remained alive among zooplankton retained by 202-μm-mesh nets from the small-pond stock.

Zooplankton from the small pond that were retained by a 202-μm-mesh net were harmful to walleye larvae. Subsamples of water from the small pond contained 95.3–98.5% *Diacyclops thomasi* and 1.5–4.7% *Daphnia rosea*. The former species, a cyclopoid copepod, was implicated in predation on Lake Michigan fish larvae (Hartig et al. 1982; Hartig and Jude 1984). It is known that mortality from predation on fish larvae increases as the concentration of cyclopoids increases (Davis 1959; Fabian 1960; Sukhanova 1968). Densities of copepods as low as 100/L are known to be harmful to fish larvae.

First Food of Larvae

Freshwater cyclopoids form an important part of the food of many fish larvae including walleye (Houde 1967; Siefert 1972; Bulkley et al. 1976). Therefore, we wanted to know what zooplankton from the small pond were used as a first food. The copepods consisted mainly of copepodites and adults. The percentages of larvae that contained food in their guts were 43.6% for the Fox River stock ($N = 55$) and 21.6% for the Little Cutfoot Sioux Lake stock ($N = 134$). Total lengths of walleye larvae at first feeding were 8.9 mm for the Fox River stock (range, 8.3–9.9 mm) and 9.1 mm for the Little Cutfoot Sioux Lake stock (range, 8.5–10.0 mm). Both copepods and cladocera were consumed as an initial food by larval walleyes (Figure 1); copepods were the most abundant food consumed; examination of Figure 2 reveals that they were dominant in the samples offered as food at this time. Walleye larvae showed positive selection for zooplankton particles 600–1,000 μm for the Fox River stock and 600–1,100 μm for the Little Cutfoot Sioux Lake stock (Table 2). Small particles consumed were copepods and larger particles were cladocera (Figure 1). Negative selection was shown for copepods smaller than 600 μm and cladocera larger than 1,100 μm. Walleye larvae at the start of feeding did not consume particles smaller than 500 μm total length even though they were present. They did not consume particles larger than 1,100 μm; however, larger forms were not very common (Table 2, Figure 2). The proportions of prey in various size categories in the fish stomachs were significantly different from the corresponding proportions in the food offered ($P = 0.006$, Lower Fox River stock; $P = 0.0349$, Little Cutfoot Sioux Lake stock).

Larval walleyes are known to first feed upon available copepods, cladocera, and other plankton within the size range of 400–1,200 μm. The taxa selected initially by walleyes are variable (Colby et al. 1979). First food of larval walleyes consisted

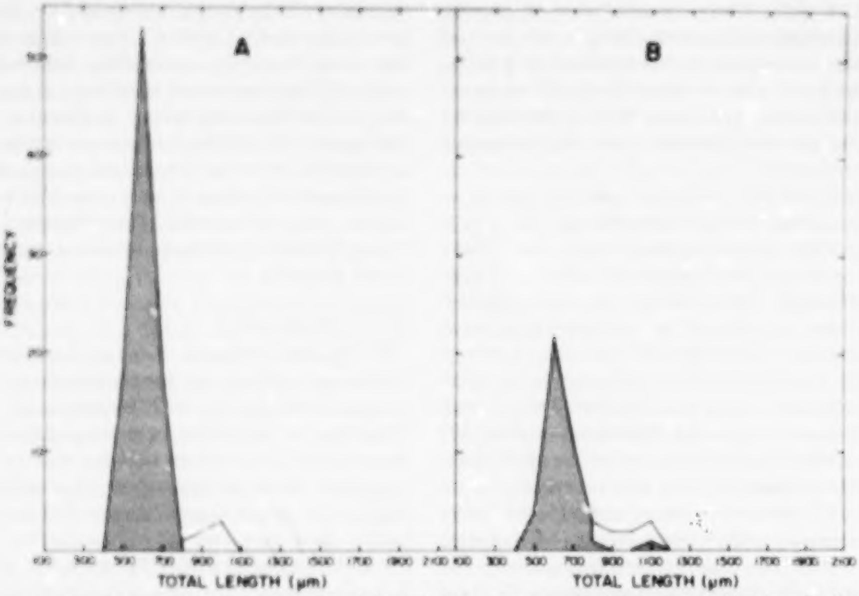

FIGURE 1.—Size frequencies of copepods (shaded) and cladocerans (unshaded) consumed by first-feeding larval walleyes from (A) Lower Fox River, Wisconsin, May 11, 1983; and (B) Little Cutfoot Sioux Lake, Minnesota, May 15, 1983.

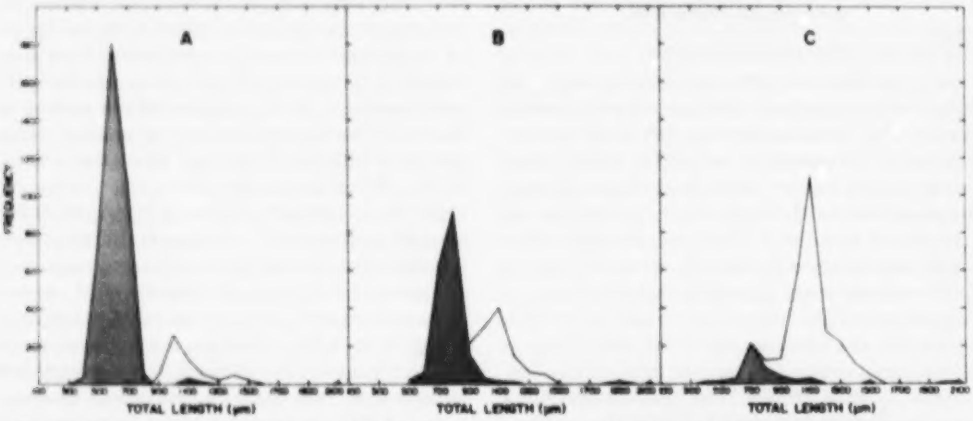

FIGURE 2.—Size frequencies of copepods (shaded) and cladocerans (unshaded) offered to larval walleyes in a growth study: (A) plankters concentrated by 202–505-µm-mesh screens, May 20, 1983; (B) plankters concentrated by 202–505-µm-mesh screens, May 26, 1983; (C) plankters concentrated by 333–505-µm-mesh screens, May 26, 1983.

of diatoms in Lake Erie (Hohn 1966; Paulus 1969) and rotifers in Minnesota rearing ponds (Smith and Moyle 1945); cladocerans and copepods were the initial food of walleye larvae in Clear Lake, Iowa (Bulkley et al. 1976). Mathias and Li (1982) reported that the preferred food of postlarval walleyes was *Daphnia pulex* (about 1.2-mm total length); however, *D. pulex* was the major component of the zooplankton present. Rotifers and copepod nauplii were not consumed at normal pond densities. Howey et al. (1980) found that when walleye larvae less than 2.0 cm long were

TABLE 2.—First food of larval walleye at commencement of feeding in relation to sizes of zooplankton fed. Statistics are percentage of prey size i fed (p_i), percentage of prey size i eaten (r_i), and linear selection index ($L_i = r_i - p_i$).

Statistic	Food particle size (i, µm)										
	200	300	400	500	600	700	800	900	1,000	1,100	1,200
Larvae from Fox River, Wisconsin											
p_i[a]	0.3	0.3	16.7	49.1	25.9	4.8	0.3	0.3	1.0	1.0	0.3
r_i[b]	0	0	0	19.8	55.2	18.8	1.0	2.1	3.1	0	0
L_i	0	−0.3	−16.7	−29.4	29.3	14.0	0.7	1.7	2.1	−1.0	−0.3
Larvae from Little Cutfoot Sioux Lake, Minnesota											
p_i[a]	0.3	1.9	12.1	28.4	41.2	8.[illegible]	1.0	1.9	2.6	1.9	0.3
r_i[b]	0	0	0	8.5	46.8	23.4	6.4	4.3	4.3	6.4	0
L_i	−0.3	−1.9	−12.1	−19.9	5.6	15.1	5.4	2.3	1.7	4.4	−0.3

[a] Total numbers of prey counted in the samples were 293 from the Fox River and 313 from Little Cutfoot Sioux Lake.

[b] Total numbers of prey consumed were 96 by larvae from the Fox River and 47 by larvae from Little Cutfoot Sioux Lake.

offered a mixed diet of daphnia and artemia, only artemia or their cysts (0.4 mm) were present in the gastrointestinal tracts. The larvae did not consume the smallest size daphnia available (0.6–1.0 mm). Raisanen and Applegate (1983) found that walleye larvae selected the cladoceran *Moina brachiata*, which was the most abundant food organism during the first days of feeding. *Cyclops vernalis* was eaten in the same proportion as it was available. *Daphnia* sp. and *Asplanchna sieboldi* were avoided. *Moina brachiata* 0.70–0.96 mm long were selected by walleyes during the first 15 d of feeding.

Diet Quality

Species lists of preferred or available food items of larval walleye do not indicate the nutritional adequacy of the diet. Therefore, we evaluated the nutritional quality of various diets for walleye larvae (Table 3). Size-frequency distributions of consumed food (Figure 1) were unavailable at the start of this experiment; therefore, size-frequency distributions of food offered are merely descriptions of available food (Figure 2). No appreciable differences occurred in percent survival of walleye larvae on various diets after 6 d (mean, 70–85% survival). However, there was considerable difference in dry weight after 6 d. Walleye larvae fed fathead minnow larvae, artemia, or zooplankton retained by 202- or 333-µm-mesh screens averaged 4.00 mg, 3.48 mg, 2.23 mg, and 1.92 mg dry weight, respectively. The rate of biomass change averaged 20.2, 18.7, 11.2, and 5.7%/d for each respective treatment. The rates for the fathead minnow and artemia diets were not significantly different; however, the zooplankton diets yielded significantly poorer growth than the other treatments ($P = 0.05$).

The results are somewhat surprising in that both sizes of zooplankton were inferior in diet quality to artemia and fish larvae. This may explain the poor results of previous investigators who used zooplankton as an initial food for larval walleyes. Our results suggest that the taxa and size composition of the zooplankton are important attributes influencing both growth and survival of larvae. The artemia we used as food were small and represented the lower end of the size distribution of selected food items. The zooplankton in the diet study were those selected by the larvae in the feeding trials. The limited sizes and varieties of available zooplankton may have contributed to the relatively poor growth response of larvae fed zooplankton. Only one zooplankton density was offered to walleye larvae (optimal density found by Li and Mathias 1982); it is possible that each zooplankton stock has its own optimum density for growth and survival of walleye larvae. It is pos-

TABLE 3.—Biomass changes and survival of postlarva-II walleyes fed one of four diets for 6 d; A and B represent progeny of different females.

Diet	Biomass change (%/d)[a]			Survival (%)	
	A	B	Mean[b]	A	B
Fathead minnows[c]	20.0	20.5	20.2 z	80	80
Artemia	21.9	15.4	18.7 z	100	70
Zooplankton[d]					
202–505 µm	14.0	8.4	11.2 y	100	70
333–505 µm	7.3	4.1	5.7 x	80	60

[a] Initial weights: A larvae, 0.974 mg; B larvae, 0.925 mg. There were 10 larvae per aquarium.

[b] Means without a letter in common are significantly different (Duncan's new multiple-range tests; $P \le 0.05$).

[c] Newly hatched; mean dry weight, 0.109 mg; mean total length, 6.04 mm.

[d] Dimensions are screen mesh sizes used to concentrate zooplankton.

sible that maximum rations of fathead minnows and artemia were provided but not of zooplankton.

Optimal Culture Conditions

Survival probability of walleye larvae reared under optimal conditions of diet, temperature, light, space, and density was excellent (Table 4). The mean survival of walleye larvae in the prolarva phase of development was 97%. During the first days of feeding, when cannibalism was known to be most intense, mean survival was 90% in the postlarva-I phase and 93% in the postlarva-II phase. Mean survival was lowest in the juvenile phase of development (88%). Because cannibalism is lowest in the juvenile phase of development (Li and Mathias 1982), we suggest that the mortality in our aquaria was due to other factors. Static-test aquaria were used for walleye culture, and waste products may have built up to intolerable levels in the two aquaria with survival less than 60%; mortality ceased after the water was changed. Survival of walleye larvae for the first 3 weeks after hatching was 71% (range, 52.3–87.4%), which is a new record for the laboratory culture of the species. The surviving fish averaged 33.6 mg dry weight (range, 22.6–48.5 mg). We do not know which culture conditions contributed the most to this success, but we feel that all contributed to some degree. The food particle size could be optimized for laboratory culture of larval walleyes as was done for Atlantic salmon (*Salmo salar*; Wankowski 1979). Continuous flow-through conditions would eliminate problems we observed in the juvenile phase.

TABLE 4.—Survival probability of Chippewa Flowage walleye larvae at different intervals of ontogeny reared under optimal conditions.

Cohort[b]	Interval of ontogeny[a]				Overall survival probability[c]
	Prolarva (P_1)	Postlarva I (P_2)	Postlarva II (P_3)	Juvenile (P_4)	
1	0.88	0.82	0.97	0.91	0.64
2	1.00	0.94	1.00	0.92	0.87
3	0.98	0.89	1.00	1.00	0.87
4	0.95	0.94	0.90	0.76	0.61
5	0.99	0.93	0.87	0.96	0.77
6	1.00	0.91	0.82	0.71	0.52
Mean	0.97	0.90	0.93	0.88	0.71

[a] Described by Li and Mathias (1982). Data are based on 100 P_1, 80 P_2, 30 P_3, and 20 P_4 larvae.

[b] Each cohort came from a different female.

[c] Product of P_1–P_4 survival probabilities.

Sufficient food and higher temperatures may help suppress cannibalism. Cuff (1977) concluded that greater food availability significantly reduced the amount of cannibalism among walleyes less than 20 d old. He observed that the presence of abundant food can inhibit cannibalism, irrespective of density levels. Feeding walleye larvae food with a high energy content may also suppress cannibalism. It is known that percids, as they develop, switch from zooplankton to larger food, including fish, to maintain food conversion efficiency at a high level (Kerr and Ryder 1977). Walleyes start to feed on fish when they reach 30–50 mm total length (Smith and Pycha 1960; Forney 1966; Bulkley et al. 1976). Although walleye larvae 7.0–12.0 mm consume fish larvae, the proportion of cladocera and fish increased in the diet of larger larvae (Houde 1967). Higher temperatures may also enhance the avoidance reactions of larval walleyes. Temperatures above 20°C tend to increase survival of walleye larvae irrespective of diet (Olson 1974; Smith and Koenst 1975).

Artemia may be the best choice of first food because it supports high growth and survival of larval walleyes without the predation impact imposed by copepods. Howey et al. (1980) observed 43.5% survival of walleye larvae through 3 weeks after swim-up on a diet of artemia supplemented with daphnia. However, larva density was less than optimal under their intensive culture conditions. More recent testing and observations in our work indicate that raising the water temperature to 25°C and feeding the larvae decapsulated artemia can increase both growth and survival rates during the first 3 weeks. Commencement of first feeding by larval walleyes occurred 2 d after hatch at 21.8°C, 3 d sooner than in the study conducted by Li and Mathias (1982) at 18–20°C and with 1.2-mm daphnia as food. This may be attributed in part to the higher temperatures in our study but also to the smaller sized artemia we used as initial food. Nevertheless, the combined influence of higher temperature and smaller food organisms had a positive influence on the energy balance of walleye larvae in our studies. Walleye larvae are free-swimming from hatch; therefore, it is important to satisfy their energy demands as quickly as possible to minimize cannibalism.

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Effectiveness of Blue Tilapia as a Weed Control Agent in Ponds

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Abstract.—Blue tilapia (*Tilapia aurea*) stocked at densities of 500 to 2,500 adults/hectare, plus their offspring, successfully controlled submersed vegetation dominated by *Najas* and *Chara*. Vegetation control resulted in higher and more uniform water temperatures and dissolved oxygen concentrations than occurred in control ponds.

Many small ponds have chronically low water quality during warm weather. Usually the low water quality results, in part, from excessive growth of aquatic plants that reduces oxygen-producing phytoplankton (Boyd 1979) and inhibits water mixing by blocking wind-induced circulation (Rottman 1977).

Water quality problems in ponds often necessitate vegetation control by either mechanical removal or herbicide treatment (Stickney 1979; Haller et al. 1980). Recently biological control, mainly through the use of plant-eating fish, has become a viable alternative (Avault 1965; Butler et al. 1968). In the 1960s, *Tilapia* spp. (principally blue tilapia [*Tilapia aurea*]) were investigated for pond-weed control (McBay 1961; Shell 1962; Childers and Bennett 1967). However, with the widespread use of grass carp (*Ctenopharyngodon idella*), research on tilapias as weed control agents was largely abandoned.

In the mid-1970s, we became interested in the potential use of tilapias to control aquatic vegetation because the introduction and use of grass carp were prohibited by law in Oklahoma at that time. Tilapias did not cause the concern that grass carp did because they generally could not survive the winter temperatures, and they were already present in Oklahoma in bodies of water reeiving thermal effluent.

Methods

Field studies were conducted during 1981 at nine experimental ponds located 12 km west of Stillwater, Oklahoma. All ponds were 0.1 hectare in area and the depth of each pond increased gradually from 0.5 m at the edge to 1.3 m at the deepest part (mean, approximately 1.0 m). Water level was maintained by periodic water input from Lake Carl Blackwell. Inlet pipes were covered with 3-mm-mesh wire screen to prevent entry of wild fish. The ponds were dry during the winter and were filled in the spring, approximately 1 month prior to use.

Aquatic macrophytes and filamentous algae were sampled monthly from May through October 1981. Flag markers along the bank were used to divide each pond into 1-m^2 sections and eight randomly chosen sections in each pond were sampled at midmonth. Vegetation was sampled by driving a 0.15-m^2 sheet-metal tube into the sediments (once per section) and removing rooted and floating vegetation within the tube. Emergents were not sampled because they composed an insignificant proportion of the total vegetation. Samples were refrigerated at 1°C then washed to remove mud, detritus, and invertebrates. Wash effluent was poured through a 0.6-mm sieve and all plant fragments were returned to the sample. Plant material in small-volume samples (samples that could be separated and identified in 2 h) was identified to species. In high-volume samples (samples that required longer than 2 h to separate and identify), species composition was estimated by selecting a random subsample (20% by weight of the total sample) and identifying the plant material to species. All samples were dried in a forced-air drying oven at 55°C for 144 h then weighed to the nearest 0.01 g. The total weight of each species in each sample was calculated by direct measure or by extrapolation of subsample weight to total sample weight. Species weights obtained by subsampling were not significantly different from those obtained from separating, identifying, and weighing the entire sample.

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[1] The Unit is jointly sponsored by the Oklahoma Department of Wildlife Conservation, Oklahoma State University, and the U.S. Fish and Wildlife Service.

Plant identifications were confirmed by Ronald J. Tyrl, curator of the Oklahoma State University herbarium. Filamentous algae were not separated by taxa, but the dominant genera were identified in May, July, and September.

Temperature and dissolved oxygen (DO) were measured twice weekly in all ponds from 15 May through 8 October. Measurements were made at the surface, middepth (0.6 m), and bottom (1.2 m) of the ponds. All measurements were made between 0900 and 1100 hours. Previous observations in these ponds, which are dominated by rooted aquatic vegetation, showed that measurements taken during this time of day are representative of low-oxygen conditions (Langston University, unpublished data). Weekly measurements of surface turbidity were made from 15 May through 8 October.

On 8 May 1981, approximately 1,000 blue tilapias were obtained from Horseshoe Lake, a thermally enriched lake in Harrah, Oklahoma. On 18 May, 50 blue tilapias (500/hectare) were stocked in each of three low-density (LD) ponds, and 250 tilapias (2,500/hectare) were stocked in each of three high-density (HD) ponds. Three unstocked ponds were used for reference.

Stocking density was assigned to each pond based upon a preliminary study of mean plant density and species composition. Ponds were classified as having high, medium, or low plant density based on the dominant plant genera and density observed in 1980. Each experimental group (LD, HD, and control) comprised one pond from each category. Observations over the last several years have shown that these ponds retain similar plant densities, within these general categories, from year to year. Within each plant-density type, fish were stocked randomly at densities of 0, 500, or 2,500/hectare. Fish were batch-weighed at stocking and individually weighed and measured at harvest (20–29 October). The data were analyzed by analysis of variance and the test of least significant differences.

Results

In 1981, mean density of plants in the reference ponds was 148.6 g/m^2. Mean densities in LD and HD ponds were substantially lower than they were in the unstocked ponds (Table 1). Mean May–October vegetation densities (principally species of *Najas* and *Chara*) were significantly different among treatments.

There were significant differences among treatments (May–October) with respect to mean temperature, DO, and turbidity (Figures 1–3). Seasonal mean temperatures and DO values at all depths were significantly greater in LD ponds and HD ponds than in reference ponds, and values in HD ponds exceeded those in LD ponds. The differences among treatments increased with depth, reaching maximum differences at the bottom of

TABLE 1. — Mean ($N = 96$) plant densities (g dry weight/m^2) in each group of experimental ponds during July through October 1981.

Plant category	Stocking density (fish/hectare) 0	500	2,500
Najas guadalupensis	115.1	64.7	25.0
Chara sp.	26.7	0.8	3.8
Filamentous algae[a]	1.9	0.4	0.1
Potamogeton spp.[b]	4.9	0.2	2.1
Total vegetation	148.6	66.1	31.0

[a] Predominantly *Cladophora* sp.

[b] *P. pectinatus* and *P. nodosus*.

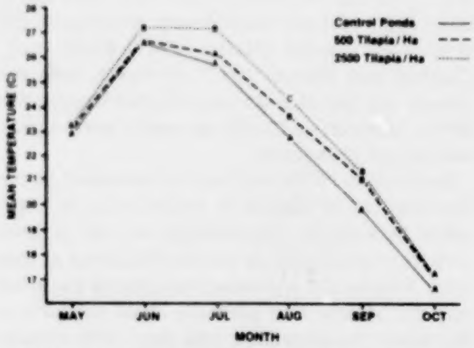

FIGURE 1. — Mean monthly temperatures (°C; means of surface, middepth, and bottom values) in experimental ponds. Monthly means designated by the same letter were not significantly different at $P < 0.05$ as determined by the least-significant-difference test. Sample sizes per treatment were 15 (May), 27 (June), 27 (July), 24 (August), 27 (September), and 9 (October).

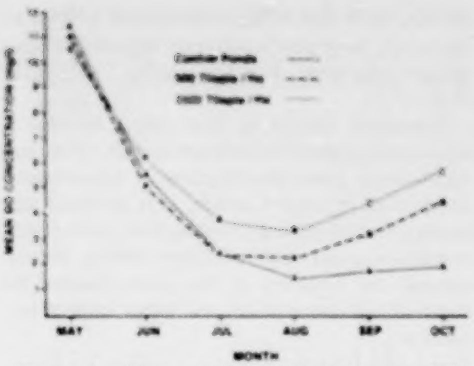

FIGURE 2.—Mean monthly dissolved oxygen (DO) concentrations (mg/L; means of surface, middepth, and bottom values) in experimental ponds. Monthly means designated by the same letter were not significantly different at $P < 0.05$ as determined by the least-significant-difference test. Sample sizes per treatment were 15 (May), 27 (June), 27 (July), 24 (August), 27 (September), and 9 (October).

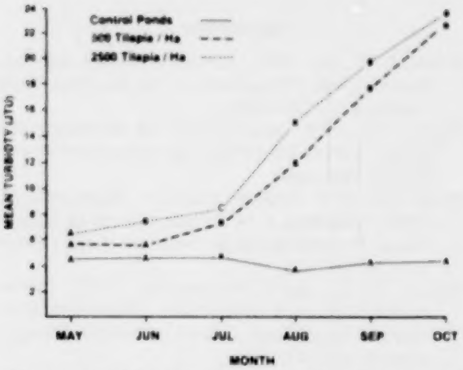

FIGURE 3.—Mean monthly turbidities (Jackson turbidity units, JTU) in experimental ponds. Monthly means designated by the same letter were not significantly different at $P < 0.05$ as determined by the least-significant-difference test.

the ponds. Mean seasonal bottom temperatures and DO concentrations in HD ponds exceeded those in reference ponds by 1.55°C and 2.24 mg/L, respectively.

The difference between surface temperature and bottom temperature was significant in each of the treatment ponds (Figure 4). Seasonal surface DO concentrations exceeded 5.0 mg/L in ponds in all treatments. Large differences between surface DO and bottom DO were evident in the reference ponds, but not in HD ponds (Figure 5). There was

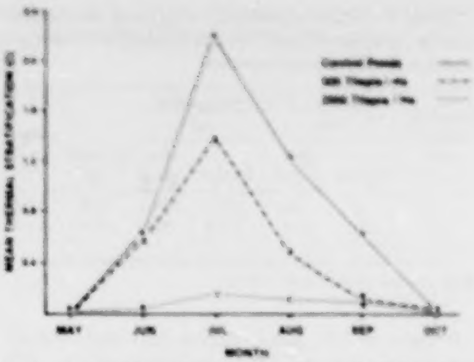

FIGURE 4.—Mean thermal differences (°C) of surface temperature minus bottom temperature in experimental ponds. Monthly means designated by the same letter were not significantly different at $P < 0.05$ as determined by the least-significant-difference test.

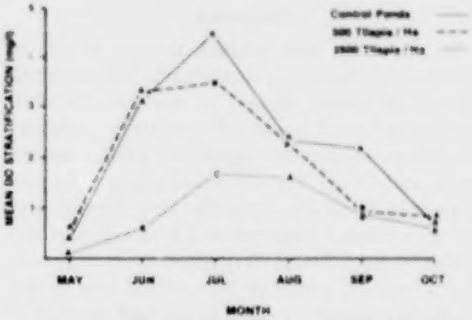

FIGURE 5.—Mean dissolved oxygen (DO) differences (mg/L) in surface value minus bottom value in experimental ponds. Monthly means designated by the same letter were not significantly different at $P < 0.05$ as determined by the least-significant-difference test.

an inverse relationship between surface and bottom thermal differences and DO concentrations and a highly significant negative correlation between mean seasonal thermal differences and both middepth ($r = -0.8529$; $P < 0.01$) and bottom ($r = 0.761$; $P = \leqslant 0.01$) DO levels of the ponds. Mean seasonal turbidity was similar in LD and HD ponds but these values were significantly lower in the reference ponds (Figure 3).

Perhaps the most important difference between treatments occurred in bottom DO levels. Anoxic bottom conditions were approached in reference ponds from July through September. Similarly, bottom DO concentrations declined to less than

TABLE 2. — Mean population variables of harvestable-size blue tilapias stocked in ponds at high (2,500/hectare) and low (500/hectare) densities.

Stocking density (fish/hectare)	Number of fish		Weight/fish (g)[a]		Growth[a] (g)	Survival[a] (%)
	Stocked	Harvested	Stocked	Harvested		
500	50	46	236.7	328.6	91.9	92.0
2,500	250	218	[illegible]	328.9	87.4	87.3

[a] Values did not differ significantly between ponds stocked at high and low densities ($P > 0.05$).

1.0 mg/L in LD ponds during July and August. Minimum bottom DO concentrations in HD ponds also occurred during July and August, but monthly means exceeded 2.4 mg/L throughout the study.

Stocking density had little effect on the production of blue tilapias during the year (Table 2). Mean weight gain exceeded 90 g/fish in all but one pond.

Discussion

In this study, blue tilapias, stocked at 500 or 2,500 adults/hectare in small ponds, plus their offspring (estimated density at harvest, 100,000/hectare in both LD and HD ponds), successfully controlled submersed vegetation dominated by *Najas* and *Chara*. The speed and degree of control were proportional to initial stocking density, with effective control observed in LD ponds and HD ponds within 120 and 90 d, respectively. The control of aquatic plants by blue tilapia seen in this study was probably related to the high survival of stocked adults plus high reproductive success and lack of predation. Pierce and Yawn (1965) and Childers and Bennett (1967) reported excellent vegetation control by Mozambique tilapia (*Tilapia mossambica*)—standing crops of 26,745 fish/hectare and 1,976 fish/hectare, respectively—in predator-free farm ponds, but they found control substantially reduced when predation by largemouth bass limited standing crops.

Control in our study appeared to result from uprooting and deleafing of plants and from reduced light penetration caused by turbidity. During the summer months, large floating beds of uprooted *Najas* were evident in all ponds stocked with blue tilapias. The leaves had been stripped from many of the plants in these floating beds. Lahser (1967) has previously reported that leaves are removed by Mozambique tilapia to obtain attached periphyton. Increased turbidity levels, such as observed in both LD and HD ponds and the corresponding reduction of light penetration, which resulted from uprooting of plants and nest building by fish, have previously been reported to cause reduced vegetation densities (Lahser 1967; Noble et al. 1976).

Vegetation control by blue tilapia resulted in significantly higher temperature and DO levels and significantly lower stratification in experimental ponds than in control ponds. It is probable that increased wind mixing resulting from reduction of vegetation is responsible for these effects, because natural clay turbidity in the ponds limited the depth of light penetration and hence oxygen production.

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Food Habits of Small Hatchery-Reared Florida Largemouth Bass

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Abstract.—Linear selection indexes of zooplankton prey taxa (L_i) were calculated for 250 hatchery-reared Florida largemouth bass (*Micropterus salmoides floridanus*). Fish began feeding at a mean total length of 6.7 mm and exhibited positive selection for copepod nauplii. Sequentially, selection shifted to copepod adults for 7.5–10.5-mm fish, to adult copepods and cladocerans for 10.9–20.8-mm fish, and, lastly, to predominantly immature insects for 22.8-mm fish. Although selection values were negative for 7.5–10.5-mm fish feeding on cladocerans, 30% more cladocerans than copepod adults were consumed. No rotifers were found in gut contents of fish even though they were present in high numbers in ponds.

The purpose of this paper is to describe the feeding habits of small (<25-mm total length) Florida largemouth bass (*Micropterus salmoides floridanus*) in hatchery ponds located in north central Texas at the Texas Parks and Wildlife Department's Fort Worth Hatchery. Few studies on the food habits of young largemouth bass in hatchery ponds have been published. Cooper (1936) reported the food habits of young largemouth bass in Michigan hatchery ponds based on gut contents. Rogers (1968) studied food habits of young hatchery-reared largemouth bass in four Alabama rearing ponds but only briefly mentioned plankton samples taken during the fish collection period. During this study, we placed particular emphasis on examining the relation between the composition and abundance of available crustacean zooplankton prey and predation by young hatchery-reared largemouth bass.

Methods

Three dry earthen ponds ranging from 0.31 to 0.36 hectare (mean, 0.32) were partly filled to approximately one-third capacity and treated with inorganic fertilizers (liquid phosphoric acid at 1 mg/L of 54% P_2O_5 and liquid 32%-N ammonium nitrate at 0.5 mg/L of NH_4NO_3) and inorganic fertilizer (224 kg/hectare of cottonseed meal, 41% protein) (Boyd 1979; Geiger 1983). Supplemental applications of organic fertilizer (45 kg/hectare of cottonseed meal) and inorganic fertilizers (same types and rates as initial treatment) were applied to ponds two or three times weekly until 10 or 14 d before ponds were harvested.

One day after it received water, each pond was inoculated with approximately 6,000 *Daphnia* (cf. *Daphnia rosea*) collected from a zooplankton nursery pond. Ponds were filled to capacity on 5 April 1984, 7 d after puddling. Adult Florida largemouth bass were stocked at the rate of 84 kg/hectare on 6 April. Incoming water was filtered through saran sock filters to prevent the introduction of wild fish.

Zooplankton populations were sampled with the pump and boom procedure described by Farquhar and Geiger (1984) beginning 1 d after ponds were filled and then every Monday, Wednesday, and Friday during a 33-d culture period. Water (30 L) collected from the pond kettle area at 0700–0800 hours was poured through an 80-μm-mesh Wisconsin plankton net. Zooplankters were preserved in a solution of cold 5% buffered formalin–sucrose (Haney and Hall 1973). Crustacean zooplankton density was estimated by counting three 1-mL aliquots taken from a 100–200-mL concentrate. Organisms were identified under a binocular dissecting microscope to the lowest practical taxa (Ward and Whipple 1959) and expressed as numbers per liter.

Fry became available in all ponds 10 d after broodfish stocking. They were collected on the same day as zooplankton with a seine net over a 31-d period ($N = 230$ fish). Ten fish of approximately equal size were collected from each pond and preserved in 10% formalin. Total length (TL) of preserved fish was measured and the entire digestive tract (gut) of each fish was removed and flushed into a Sedgewick–Rafter cell, where the contents were identified and enumerated. Linear selection indexes of prey taxa (Strauss 1979) were calculated as $L_i = r_i - p_i$, where r_i = frequency of occurrence of prey taxon i in guts and p_i = frequency of occurrence of that taxon in the respective ponds from which fish were sampled.

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Results and Discussion

Succession patterns and species composition of crustacean zooplankton in the three ponds followed similar patterns. The data presented here are mean values for all ponds (Figure 1) and are intended only to show general trends of crustacean abundance. Major zooplankton life stages in the ponds were copepod nauplii and adults (calanoid and cyclopoid species) and the cladoceran genera *Bosmina*, *Daphnia*, and *Ceriodaphnia*. Because of difficulties experienced in identifying cladoceran genera from gut samples, these three genera were collectively termed cladocerans for further analyses. Rotifers were present in the ponds throughout the study but were omitted from data analyses because they were not consumed by largemouth bass. Adult copepods reached maximum densities first, 12 d after broodfish stocking. Copepod nauplii peaked on days 17 and 26, the greatest density occurring on day 26. Cladocerans reached maximum densities on day 21.

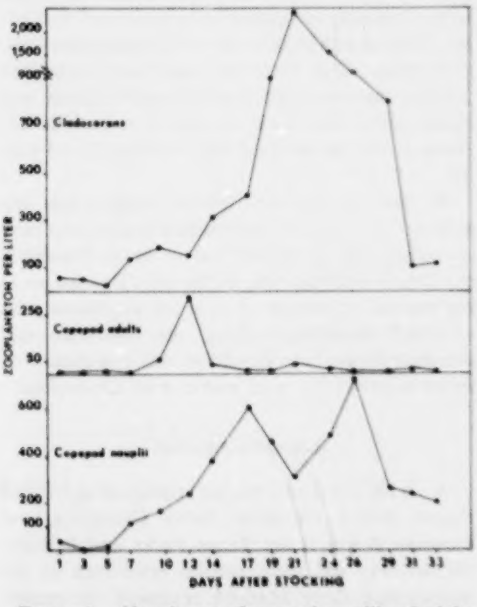

FIGURE 1.—Abundances of copepod nauplii and adults and cladocerans over a 33-d period in spawning–rearing ponds for Florida largemouth bass.

Fish 4.5–5.5 mm TL were collected from nests and their guts contained no food items. Fish began feeding at 6–7 mm TL (mean, 6.7 mm) and these fish strongly selected copepod nauplii over adult copepods and cladocerans (Table 1); nauplii made up 11% of the zooplankton numbers in ponds but formed 94% of the total food items in guts. Nauplii continued to be present in fish guts until fish reached 14–15 mm (mean, 15.5 mm) and reoccurred in the 22–23-mm (mean, 22.8 mm) fish. Selection indexes for nauplii were always negative once fish grew past 7 mm (Table 1).

No rotifers were found in any fish even though they were always present in ponds. Rogers (1968) reported rotifer remains in the stomachs of all but two size groups (5–40-mm TL) of largemouth bass ($N = 525$) from Alabama hatchery ponds. He stated that rotifers were abundant throughout the study period, which might suggest that they are preyed upon by small largemouth bass more than previously believed. Perhaps rotifers might be preyed upon if more preferred, and probably more proteinaceous, prey such as crustacean zooplankton are scarce.

Fish 7.5–10.5 mm TL exhibited positive selection for adult copepods and negative selection for cladocerans (Table 1). During this time, copepods averaged 27% of the total food items in fish guts but only 1–2% of the crustaceans in ponds, whereas cladocerans averaged 60% of gut contents and 72% of pond crustaceans. This suggests a preference for adult copepods over the most abundant prey group.

Fry 10.9 mm TL and larger showed positive selection for both copepod adults and cladocerans (Table 1). Selection values were usually greater for cladocerans than copepods. Copepods made up 1–28% (mean, 11%) of the total numbers in the gut samples of fish 10.9–22.8 mm TL and 0–4% (mean, 1%) of the crustacean zooplankton in ponds. Cladocerans made up 65–90% (mean, 73%) of the numbers of prey in gut samples and 26–69% (mean, 47%) of zooplankton populations.

Immature insects first appeared in the diet of fish 11–12-mm TL (mean, 11.7 mm) and were

TABLE 1.—Relative abundances of prey taxa in guts of young-of-year Florida largemouth bass and in Texas rearing ponds, and calculated linear selection indexes (L_i)[a] by predator size.

Mean total length of fish (mm)	Copepod prey						Cladoceran prey		
	Nauplii			Adults					
	% in guts	% in ponds	L_i	% in guts	% in ponds	L_i	% in guts	% in ponds	L_i
6.7	94	11	+0.83	2	11	−0.09	4	78	−0.74
7.3	18	23	−0.05	7	1	+0.06	75	76	−0.01
7.8	19	63	−0.44	72	1	+0.71	9	36	−0.27
8.9	10	18	−0.08	49	1	+0.48	42	80	−0.38
10.2	1	8	−0.07	9	1	+0.08	90	91	−0.01
10.5	21	23	−0.02	4	1	+0.03	75	76	−0.01
10.9	10	21	−0.11	3	0	+0.03	87	79	+0.08
11.7	1	30	−0.29	26	2	+0.24	70	68	+0.02
13.0	7	56	−0.49	24	1	+0.23	70	45	+0.25
15.3	0	31	−0.31	2	0	+0.02	89	69	+0.20
16.8	0	81	−0.81	10	2	+0.08	65	17	+0.48
17.4	0	58	−0.58	19	1	+0.18	79	41	+0.38
19.1	0	62	−0.62	3	1	+0.02	88	37	+0.51
20.6	0	70	−0.70	1	4	−0.03	90	26	+0.64
22.8	3	93	−0.90	13	1	+0.12	9	5	+0.04

[a] $L_i = r_i - p_i$; r_i is the decimal proportion of prey i in fish guts and p_i is the proportion of taxon i in the environment.

found in guts of all remaining size groups. Overall percentage, however, was low (<10%) until fish reached 22–23 mm (mean, 22.8 mm). At 22.8-mm TL, fish appeared to shift from predominantly crustacean zooplankton (91%) to immature insects (73%). This contrasts with Rogers' (1968) finding that hatchery largemouth bass fry fed primarily on midges after reaching 15 mm TL. During the present study, crustacean zooplankton prey were still readily available when fish were 15 mm, but had decreased to an overall mean density of approximately 100 organisms/L by the time fish reached 22.8 mm. This reduction of available zooplankton prey may account for the switch to insect prey. Cannibalism was noted only once: a 23-mm fish contained a 10.3-mm largemouth bass.

In summary, our data show that nauplii were the most important dietary component as first-feeding largemouth bass left the nest. Strauss's (1979) linear index model indicated that fish 7.5–10.5 mm TL selected against cladocerans and for copepod adults. We feel this information is misleading because it ignores numerical (or biomass) intake. While copepods may provide more energy on a dry-weight basis than cladocerans (*Diaptomus siciloides*, 5,468 cal/g; *Daphnia pulex*, 4,478 cal/g: Comita and Schindler 1963), largemouth bass 7.5–10.5 mm TL consumed 30% more cladocerans than copepods. Young largemouth bass are strike-feeders, relying on sight to capture a prey item. Probably the deeper-bodied, slower, constantly moving cladocerans were more easily seen and captured than the swifter, often intermittently moving copepods (O'Brien 1979; Mills et al. 1984). Moreover, cladocerans have been found to be much less able than copepods to avoid attack from a predator; copepods have demonstrated great success at evading predator attacks (Szlauer 1965; O'Brien 1979). Thus, in spite of negative selection values, cladocerans were probably more easily captured than copepod prey and were extremely important prey items to 7.5–10.5-mm largemouth bass in terms of biomass and caloric intake. Fish 10.9 mm and larger exhibited positive selection for both copepod adults and cladocerans. Insects did not appear to become important food items until fish reached 22–23 mm TL.

We believe that these results suggest that the management practice of fertilizing largemouth bass spawning–rearing ponds just prior to broodfish stocking is well founded. By the time fry start leaving the nest in search of food (approximately 8–14 d after broodfish stocking), the crustacean zooplankton forage base should be well established in terms of prey type, size, and overall abundance.

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Dietary Androgen-Estrogen Combinations in Growth Promotion in Fingerling Rainbow Trout[1,2]

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Abstract.—Eight semipurified diets containing various combinations (totaling 2.0 mg/kg diet) of the androgens 17α-methyltestosterone (MT) and 5α-dihydrotestosterone (DHT) and the estrogen estradiol-17β (ES) were fed to four replicate groups of rainbow trout (*Salmo gairdneri*) fingerlings (initial weight = 0.7 g) for 10 weeks to determine relative growth-promoting effectiveness. Fish fed MT alone or in combination with DHT gained 27.3% more body weight than controls ($P < 0.05$). Estradiol alone or in combination with DHT decreased body weight gain ($P < 0.05$) and inclusion of the estrogen in diets containing MT suppressed ($P < 0.05$) the anabolic effectiveness of this androgen. Dihydrotestosterone had no effect ($P > 0.20$) on body weight. Feed conversion (weight of feed fed/fish weight gained) and average weight gain were recorded over bimonthly periods to examine the effects of treatments on these characteristics. Methyltestosterone enhanced growth ($P < 0.05$) without changing feed utilization. Decreases ($P < 0.01$) in average weight gain were associated with increases ($P < 0.05$) in feed conversion of fish fed ES-treated diets, suggesting a negative effect of estrogen treatment on feed utilization or appetite at the levels used.

Dietary androgenic steroid supplements are more effective growth promoters for juvenile salmonids than estrogenic compounds (Donaldson et al. 1979; Higgs et al. 1982). Emphasis on androgen treatment, however, may obscure the potential importance of both hormones in regulating the growth response. Maximum gains in postpubertal beef cattle are obtained by differentially treating sexes (Heitzman 1976, 1979) to optimize circulating androgen and estrogen levels (Heitzman 1976). In young calves, androgen–estrogen combinations have produced greater growth responses than single hormone applications (Vander Waal 1976). Similar treatment of young salmonids with dietary androgen–estrogen combinations might enhance growth beyond that obtained by androgen treatment alone. Furthermore, such combinations could optimize the treatment of a stock since males and females may be affected differently.

The present study was designed to examine the growth responses of fingerling rainbow trout (*Salmo gairdneri*) fed semipurified diets containing various combinations of the androgens, 17α-methyltestosterone and 5α-dihydrotestosterone and the estrogen, estradiol-17β. 17α-Methyltestosterone (MT) is an effective growth promoter for juvenile salmonids (Donaldson et al. 1979; Higgs et al. 1982) and has produced gains in rainbow trout fed levels between 1.0 and 12.5 mg/kg diet (Simpson 1976; Fagerlund and McBride 1977). Estradiol-17β (ES) is moderately anabolic in coho salmon (*Oncorhynchus kisutch*) fed 2.5 mg/kg diet (Yu et al. 1979), but retards the normal growth of trout fed 5.0 (Sower et al. 1983) and 20.0 mg/kg diet (Johnstone et al. 1978). 5α-Dihydrotestosterone (DHT) is a nonaromatizable androgen (Mainwaring 1976) that is not considered important in the proliferation (Powers and Florini 1975) or growth (Mainwaring 1976) of skeletal muscle. Potential anabolic effects of this steroid have not been examined for salmonids.

Materials and Methods

Eight semipurified[3] diets were prepared, seven containing various combinations of MT, ES, and DHT at 2.0 mg/kg diet and a control containing no steroid supplementation. Diets containing a single steroid preparation received 2.0 mg steroid/kg diet. Diets containing two steroids were formulated on a 1.0:1.0-mg/kg basis. The MT:DHT:

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[3] Modified from the standard NRC (1978) test diet for cold-water fishes containing 32.79% casein, 14.13% gelatin, 28.15% dextrin, 11.48% alpha-cellulose, 6.45% soy oil, 2.00% cod-liver oil, 4.00% mineral mix, 0.55% vitamin mix, and 0.45% choline chloride.

ES diet contained 0.5:0.5:1.0 mg steroid/kg diet, respectively, to maintain a 1:1 androgen : estrogen regimen in this combination. Respective steroid–soybean oil stock solutions (1 mg/g) were initially prepared by dissolving each steroid in 10 mL ethanol and then adding the ethanol to the oil. Appropriate amounts of each solution replaced a similar amount of the soybean oil component used in formulating each diet. All diets were mixed, dried, and stored as outlined by Garling and Wilson (1976).

Twenty fish (5 months old, 0.7 g initial weight) were contained in each 110-L flow-through aquarium supplied with a water flow of 1 L/min and continuous aeration. Water temperature was a constant 11.5°C. Overhead fluorescent lighting was set on a 14-h-light : 10-h-dark regimen. Four tank replicates were used per treatment. Mean fish weight of each unit was obtained by dividing the total weight of fish in each tank by the number of fish in each tank.

Tanks of fish were given dry feed at a rate of 4.0% of the total wet body weight in each unit daily, divided into two equal feedings (1000–1100 and 1600–1700 hours) for 10 weeks. The 4.0% feeding rate appeared to exceed the immediate needs of the fish since uneaten feed collected in all tanks after each feeding. Feeding rates were adjusted every 2 weeks and when mortalities occurred. Fish were not replaced in the units when deaths occurred. Mortalities were less than 1.0% and were independent of type of diet fed.

A split-plot analysis of variance (ANOVA) was used to examine the effects of treatments through time on average weight gain and feed conversion (weight of feed fed/fish weight gained). The validity of assuming equal variance–covariance structure from treatment to treatment and period to period was ensured by using conservative critical values (Gill 1978). Tukey's and Dunnett's comparisons were applied where appropriate.

Body composition was analyzed by standard AOAC (1970) methods. Five fish from each replicate group were pooled for analysis. Values obtained represented the mean of two determinations from each replicated group. The effects of treatment on body composition were analyzed with a nested ANOVA and Tukey-type and Scheffé comparisons.

Results and Discussion

With mammals, the advantage of using androgen–estrogen combinations to stimulate growth is based upon finding appropriate treatment levels which maximize the growth-promoting potential of each steroid (Heitzman 1976, 1979). Since separate growth-promoting mechanisms are elicited by each hormone (Heitzman 1979), the additive effects enhance growth beyond that which can be obtained by a single hormone preparation. The levels of ES used to treat the rainbow trout in our study, either alone or in combination with DHT, depressed growth ($P < 0.05$, Table 1) and did not permit adequate examination of combinations to promote growth. Further, when ES was combined with levels of MT that otherwise stimulated growth, the anabolic response that occurred with this androgen was suppressed ($P < 0.05$). Fish fed MT and ES, or MT, DHT, and ES, gained the same weight as controls. Estrogenic compounds are generally thought to decrease the growth of salmonids when administered even at doses as low as 1 mg/kg diet (Higgs et al. 1982), although Yu et al. (1979) reported that a 2.5-mg/kg dose of ES was anabolic in coho salmon. Our results indicate that growth-depressing levels of ES also negate the growth-stimulating effects of MT when administered in combination with this androgen.

Methyltestosterone fed at 2.0 mg/kg diet enhanced the average weight gain of treated fish relative to controls by 27.3% ($P < 0.05$) after the 10-week feeding period in our study (Table 1). This percentage gain is comparable with those resulting from other low-level MT treatments of various salmonids (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1975a, 1977; Higgs et al. 1977; Fagerlund et al. 1979a, 1979b, 1980). A similar gain was observed in fish fed the MT:DHT combination diet ($P < 0.05$); however, due to the

TABLE 1.—Mean average weight gain (g/fish) of four replicate groups of rainbow trout fingerlings (initial weight, 0.7 g) fed steroid-supplemented diets. Values without a letter in common are significantly different ($P < 0.05$) by Tukey's comparison of means (SE of means = 0.1 g).

Dietary steroid composition[a]	Respective steroid supplement (mg/kg diet)	Average weight gain per fish (g)
MT	2.0	7.0 z
MT:DHT	1.0:1.0	7.0 z
MT:DHT:ES	0.5:0.5:1.0	5.9 y
MT:ES	1.0:1.0	5.8 y
Control	0.0	5.5 y
DHT	2.0	5.4 yx
DHT:ES	1.0:1.0	4.9 xw
ES	2.0	4.6 w

[a] MT = 17α-methyltestosterone; DHT = 5α-dihydrotestosterone; ES = estradiol-17β.

ineffectiveness of DHT fed alone ($P > 0.2$), the response was probably a result of the MT alone. Dihydrotestosterone also did not alter the growth-depressing effects of ES, but the percentage whole-body protein was increased ($P < 0.05$) in fish fed DHT combined with MT or ES (Table 2).

The ineffectiveness of DHT alone to promote growth meant, in effect, that rainbow trout were simply fed graded levels of MT (2.0 mg MT/kg diet for the MT supplement; 1.0 mg MT/kg for the MT:DHT diet). Similarity in growth rates of fish fed 2.0 and 1.0 mg MT/kg may have resulted from an overlap in biologically significant dosages. Fagerlund and McBride (1977) also observed no significant differences in the growth-promoting effects of MT between 1.0 and 3.0 mg/kg diet in experiments with steelhead (anadromous *Salmo gairdneri*).

The increases in average weight gain over that of controls ($P < 0.01$) obtained with the MT or MT:DHT treatments, first noted during weeks 6–8, were not associated with significant changes in feed conversion within these periods, with one exception (Tables 3, 4). This indicates that, while steady declines in feed conversion occurred (though these were insignificant, $P > 0.20$, except in weeks 6–8 for MT:DHT-treated fish), an improvement in feed utilization was not the primary cause for increased weight gain observed in MT-treated fish after 8 weeks. A combination of small, insignificant ($P > 0.20$) gains in weight and increased amounts of feed determined by the fixed, weight-related feeding schedule over 8 weeks contributed more to the greater gain. Methyltestosterone-treated fish again gained more body weight ($P < 0.01$) than controls during weeks 8–10 because they were larger and were fed more, and not because their feed conversion was significantly ($P > 0.20$) improved. Previous authors (Fagerlund et al. 1979a, 1979b, 1980) have reported improvements in feed conversion after long-term, low-level MT treatment. However, these changes were also associated with increased feed intake of fish fed on a satiation basis. This raises the question whether the increased gain observed in these studies was due primarily to increased feed utilization, increased intake, or both. Our results indicate that the short-term (2 and 10 weeks) increase in weight gain of MT-treated fish is not caused by a significant improvement in feed utilization.

In contrast, decreased ($P < 0.01$) weight gain of ES-treated fish (weeks 8–10) was associated with increased ($P < 0.01$) feed conversion. This was due to a decreased intake of presented feed or a decreased tissue use of absorbed nutrients (or both), which slowed growth. Decrease in feed intake of ES-treated ovarectomized rats results from an increase in triglyceride availability for metabolic fuel (Roy and Wade 1977) caused by a hormone-induced mobilization of body fat stores (Watkins et al. 1972; Kim and Kalkhoff 1975; Roy and Wade 1977; Ferreri and Naito 1978). Since fat content was not changed ($P > 0.20$) in our rainbow trout (Table 2), a metabolic control of feed intake was not suggested. The levels of ES used were probably

TABLE 2.—Influence of experimental diets on whole body (percent wet weight) moisture, protein, lipid, and ash contents of fingerling rainbow trout. Values[a] within columns without a letter in common are significantly different (Tukey's comparison of means; $P < 0.05$).

Dietary steroid[b] composition	Moisture	Protein	Lipid	Ash
Control	78.25 *z*	11.69 *zy*	7.00 *z*	1.90 *z*
DHT	79.18 *z*	10.87 *z*	7.12 *z*	1.40 *z*
MT	79.12 *z*	11.16 *zy*	7.28 *z*	1.83 *z*
ES	78.96 *z*	11.96 *y*	6.91 *z*	1.27 *z*
MT:ES	79.10 *z*	11.54 *zy*	6.54 *z*	1.60 *z*
MT:DHT	77.29 *z*	12.07 *y*	7.57 *z*	2.00 *z*
DHT:ES	78.68 *z*	12.11 *y*	6.45 *z*	1.75 *z*
MT:DHT:ES	79.12 *z*	11.40 *zy*	6.91 *z*	1.64 *z*
SE	0.61	0.23	0.29	0.16

[a] Values represent the mean of eight determinations per treatment, two samples from each of four replicate groups (five fish per group).

[b] Supplemented as 2.0 mg/kg diet (MT = 17α-methyltestosterone; DHT = 5α-dihydrotestosterone; ES = estradiol-17β).

TABLE 3.—Average weight gain by control rainbow trout and deviations from control gain by test fish fed steroid supplements. Asterisks denote significant deviations at $P < 0.05$* or $P < 0.01$** (Dunnett's test).[a]

Dietary steroid supplement[b]	Period (weeks)				
	0–2	2–4	4–6	6–8	8–10
	Control fish: average weight gain (g)				
	0.4	0.8	1.1	1.3	2.0
	Test fish: deviation from average control gain (g)				
MT	0.1	0.1	0.2	0.5**	0.6**
MT:DHT	−0.1	0.1	0.2	0.6**	0.6**
MT:DHT:ES	0.1	0.2	0.1	0.3*	−0.3*
DHT	−0.1	0.2	0.0	0.1	−0.2
MT:ES	−0.1	0.2	0.1	0.3*	−0.2
DHT:ES	0.0	0.1	0.0	−0.2	−0.5**
ES	−0.1	0.1	−0.1	−0.2	−0.7**

[a] The mean squared error of the interaction of average weight gain and period was 0.02. The SE of the difference between two treatment means was 0.1.

[b] Supplemented as 2.0 mg/kg diet (MT = 17α-methyltestosterone; DHT = 5α-dihydrotestosterone; ES = estradiol-17β).

TABLE 6.—Average feed conversions (g dry weight of feed fed/g wet weight gain) by control rainbow trout and deviations from control conversions by test fish fed steroid supplements. Asterisks denote significant deviations at $P < 0.05$* or $P < 0.01$** (Dunnett's test).[a]

Dietary steroid supplement[b]	Period (weeks)				
	0–2	2–4	4–6	6–8	8–10
	Control fish: average feed conversion				
	0.96	0.99	0.91	1.15	1.19
	Test fish: deviation from average control conversion				
MT	−0.04	−0.08	−0.10	−0.16	−0.12
MT DHT	0.03	−0.14	−0.13	−0.20*	−0.11
MT DHT ES	−0.06	−0.13	−0.04	−0.03	0.33**
DHT	−0.01	−0.20	−0.10	−0.11	−0.03
MT ES	0.03	−0.09	−0.08	−0.08	0.17
DHT ES	0.05	0.01	0.00	0.34**	0.26*
ES	0.06	−0.07	0.03	0.23*	0.41**

[a] Mean squared error of the interaction of average feed conversion and period was 0.0125. The SE of the difference between two treatment means was 0.08.

[b] Supplemented at 2.0 mg/kg diet (MT = 17α-methyltestosterone; DHT = 5α-dihydrotestosterone; ES = estradiol-17β).

toxic to the fish and depressed their appetite. Growth-depressing levels of androgens and estrogens have also decreased appetite in treated coho salmon (Fagerlund and McBride 1975a) and goldfish (*Carassius auratus*: Yamazaki 1976). Appetite-depressing effects of high-dose gonadal steroid treatment have also been observed in mammals (Wade and Gray 1979).

In summary, results from the present study confirm the anabolic effectiveness of MT in rainbow trout, which enhanced weight gain by 27.3% after 10 weeks. Estradiol, however, depressed growth at the levels used and appeared to inhibit the anabolic action of MT when combined with this androgen. The effects of MT and ES on growth differed when examined over time; MT enhanced growth without improving feed conversion while ES depressed feed utilization or acceptance (i.e., appetite). Dihydrotestosterone had no effect on body weight gain.

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Cage-Rearing Rainbow Trout in Dugout Ponds in Eastern South Dakota

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Abstract.—Rainbow trout (*Salmo gairdneri*) were reared in cages in dugout farm ponds in east-central South Dakota during 1982 and 1983 to determine growth and survival, optimal feeding rate and stocking density, and economic feasibility. Trout had 1.9–4.2% daily weight gains and 93–100% survival. The optimal feeding rate was 3–4% of body weight daily. The high cost of fingerlings ($35/100 for 66-g rainbow trout) was the greatest expense in a hypothetical cage-culture operation, but the study demonstrated that an owner of dugout ponds could economically produce a large weight of acceptably sized rainbow trout in a relatively small cage (about 10 m^3) over a short time period.

Cold-water fisheries in South Dakota are limited, hence many residents do not have an opportunity to catch or purchase fresh trout. The majority of South Dakota lakes and streams will not support trout populations for more than a year due to winterkill and summerkill. Rainbow trout (*Salmo gairdneri*) have been reared in the prairie pothole lakes of Canada and North Dakota (Sunde et al. 1970; Lawler et al. 1974; Myers and Peterka 1976). Limited experimental rainbow trout culture has been attempted in the lakes, ponds, and dugout ponds of eastern South Dakota (Vodehnal 1982).

Approximately 100,000 dugout farm ponds have been excavated in South Dakota since the mid-1930s (Vodehnal 1982); thus they represent a major aquatic resource. Such ponds are strategically located and constructed to collect surface run-off for subsequent use by livestock, but these ponds also have other potential uses (limited irrigation, wildlife use, and recreation). Most dugouts have a surface area between 0.05 and 0.10 hectare, are usually less than 3.0 m deep, and have a high potential for winterkill and summerkill due to their shallow depth, small size, and nutrient loading from livestock and other agricultural practices. Raising annual fish crops appears to be the only feasible fish culture method.

Vodehnal (1982) demonstrated that rainbow trout could grow in dugouts. Fingerlings were stocked in the spring and harvested in the fall, but overall survival was only 2.6%. Poor survival was attributed to a combination of high water temperatures and low dissolved oxygen levels in July and August. Thus, the optimal culture period is only about 70 d, from mid-April to late June or early July.

The goals of this study were to examine the feasibility of cage-rearing rainbow trout in dugouts and to assess the potential for owners of these ponds to produce annual rainbow trout crops. Specific objectives were (1) to determine if cage-reared rainbow trout could effectively grow and survive in South Dakota dugouts, (2) to determine appropriate feeding rates and stocking densities for cage-reared rainbow trout, and (3) to evaluate the economic feasibility of rearing rainbow trout in cages on an annual basis.

Methods

Dugout ponds were selected from within Brookings County, South Dakota, in 1982 and 1983 on the basis of water depth (>2.5 m). The cages consisted of 12.7-mm-mesh plastic netting secured to a frame (0.5 × 1.0 × 2.0 m deep) of 38-mm-square pine lumber with no sharp corners protruding to the inside. Two polystyrene blocks (0.3 × 0.3 × 0.6 m long) secured to the ends of each cage provided flotation. A 3.2-mm-mesh plastic net (0.3 m deep) was attached around the top inside of each cage to retain floating feed. The removable plywood lid had a 0.25-m-square feeding port covered with 12.7-mm-mesh plastic netting. This cage design allowed adequate water circulation and a range of dissolved oxygen and temperature conditions within the depth of the cage.

Rainbow trout fingerlings (Manchester strain) were obtained in 1982 and 1983 from Cleghorn Springs State Fish Hatchery in Rapid City, South Dakota. The fish arrived in vigorous condition both years and were stocked directly into the cages; no temperature acclimation period was necessary because hauling temperatures closely matched pond-water temperatures. Fish received a pelleted floating ration (Purina Trout Chow) containing no less than 37.5% protein. They were fed once daily in the early evening; unfed fish served as controls.

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Year 1.—A randomized experimental design with three treatments (fish fed 0, 2, or 4% of body weight daily) of five replicates each was used in 1982. Three rows of five cages each were arranged in one pond; the cages were 2.0 m apart within and between rows.

Rainbow trout weighing an average of 34.5 g (146 mm total length) were stocked on 27 April 1982 at a density of 1.21 kg/m^3 (35 fish/cage), and feeding was started 4 May 1982, following an acclimation period of about 1 week. Ten fish were weighed every 2 weeks from one randomly chosen cage for each feeding rate, and rations were adjusted accordingly. The fish were fed 0, 2, or 4% of their average stocking weight daily for the first 2 weeks; thereafter, mean weights (and proportional feed requirements) were linearly projected for each day based on the daily growth increment during the previous 2-week period. The rainbow trout were fed on 47 of the 58 d and were harvested on 23 June 1982 when dissolved oxygen levels became critical (<5 mg/L) and the fish refused to feed. Dissolved oxygen was measured immediately adjacent to three cages at the surface and at 1 m, 2 m, and bottom depths by the azide modification of the Winkler method (APHA et al. 1975). Measurements were made in the early morning, usually before sunrise, when dissolved oxygen levels were expected to be lowest. Fish were eviscerated and placed in ice-filled polystyrene coolers after measurement of total lengths (mm) and wet weights (g). Food conversion (weight of food fed/weight gained by fish) and relative weight (*Wr*) values were calculated. Relative weight ($Wr = 100 \times$ actual fish weight/standard weight for a fish of the same length) was used as an index of condition (Wege and Anderson 1978).

Year 2.—A 3 × 3 factorial design with three feeding rates, three stocking densities, and five replicates each was used in 1983. Fifteen cages were placed in each of three ponds in the same manner as in 1982. Each of three stock densities, 0.94, 1.40, or 1.88 kg/m^3 (35, 52, or 70 fish/cage, respectively), was randomly assigned to one of three ponds, and each of the three feeding rates (0, 3, and 5% of body weight daily) were randomly assigned to five cages within each pond.

Ideally, 45 cages would have been required in each pond (five replicates of each of the nine density–ration treatments) to statistically distinguish pond effects on growth, but dugouts are too small to accommodate 45 cages, and the dissolved oxygen demand by 2,355 fish/pond could have jeopardized the study. Consequently, only 15 cages were used in each pond, and the assumption was made that pond temperature regimes would be similar. (Ponds subsequently developed quite different temperature profiles, lowering the power of the statistical tests of density effects.)

Rainbow trout fingerlings, weighing an average of 26.9 g (138 mm total length), were stocked 20 April 1983; approximately 30 fish died during the following week and were replaced. Feeding commenced on 23 April 1983 after a 3-d acclimation period. Mean weights and fish ration sizes were estimated in the same manner as in 1982 except that fish weights were measured from a 28% sample (10, 15, and 20 fish from stocking densities of 0.94, 1.40, and 1.88 kg/m^3, respectively) of rainbow trout every 10–12 d. Trout were fed on 60 of the 63 d and were harvested on 22 and 23 June 1983, before dissolved oxygen levels became limiting (<5 mg/L). Dissolved oxygen was measured in the same manner as in 1982. Total lengths (mm) and weights (g) were measured before the fish were eviscerated and packed in ice. Food conversions and *Wr* values were calculated.

Analysis of variance was used to detect significant differences occurring among treatments in both years. Waller-Duncan *k*-ratio *t*-tests were used to determine which treatments had significant effects.

Results and Discussion

Growth and Survival

Percent daily weight gains ranged from 1.9 to 4.2% over all treatments for both years; survival ranged from 93 to 100% (Table 1). Statistical analysis revealed significant differences in mean lengths, weights, and *Wr* values among feeding treatments in 1982 ($P \leq 0.01$) and 1983 ($P \leq 0.05$). The 1982 data indicated significant differences among all three treatments for mean length and weight, but mean *Wr* differences only existed between the unfed (control) and fed fish. In 1983, significant differences occurred in all three growth variables between unfed and fed fish (Table 1). In

TABLE 1.—Performances of rainbow trout cage-reared in east-central South Dakota dugout ponds, April–June 1982 and 1983. Values for mean final lengths and weights and mean relative weights within each stocking density (partial column) without a letter in common are significantly different ($P \le 0.05$); all food conversion values were significantly different ($P \le 0.01$).

Stocking density and feeding rate (% body weight daily)	Mean length (mm)		Mean weight (g)		Percent survival	Mean *Wr*[b]	Food conversion[c]	Percent daily weight gain[d]
	Initial[a]	Final	Initial[a]	Final				
				1982				
1.21 kg/m^3								
0	146	160 z	34.5	38.1 z	100.0	88.2 z		0.22
2	146	193 y	34.5	89.1 y	98.3	113.4 y	1.0 z	3.37
4	146	202 x	34.5	102.4 x	96.6	113.4 y	1.8 y	4.19
				1983				
0.94 kg/m^3								
0	138	136 t	26.9	21.3 t	99.4	80.3 t		−0.04
3	138	167 s	26.9	80.7 s	100.0	113.1 s	1.8 t	3.33
5	138	188 s	26.9	79.1 s	99.4	108.2 s	3.2 s	3.22
1.40 kg/m^3								
0	138	150 k	26.9	33.7 k	99.2	94.6 k		0.41
3	138	188 j	26.9	87.8 j	100.0	119.4 j	1.6 k	3.78
5	138	187 j	26.9	85.0 j	99.2	119.3 j	2.5 j	3.59
1.88 kg/m^3								
0	138	141 f	26.9	23.6 f	98.0	76.6 f		−0.22
3	138	180 e	26.9	59.8 e	94.0	93.1 e	1.5 f	2.03
5	138	179 e	26.9	58.1 e	93.4	92.8 e	2.6 e	1.92

[a] Based on a sample of 200 fish measured at the hatchery.

[b] *Wr* (relative weight) = 100 × actual fish weight/standard weight for fish of the same length.

[c] Amount of food fed/weight gained.

[d] Mean individual weight increase/mean initial weight.

addition, significant differences ($P \le 0.05$) among 1983 stock densities were reflected in the three growth variables.

Growth in one dugout (stock density of 1.88 kg/m^3) in 1983 appeared to be influenced by water clarity. Secchi-disc visibility equaled or exceeded 0.7 m from the first day of feeding to 14 May. Mean weight gain for the fed rainbow trout during this period was 1.04 g/d. Water clarity decreased from 0.9 m on 14 May to 0.3 m on 17 May, corresponding to an introduction of cattle into the adjacent pasture. Mean weight gain of the fed rainbow trout subsequently dropped to 0.24 g/d for the remainder of the study despite favorable water temperatures (14°C) and dissolved oxygen levels (>6 mg/L). Water clarity and mean weight gain in the other two study ponds remained high. DiLauro (1982) reported water clarity to be significantly lower ($P \le 0.05$) in eastern South Dakota dugouts available to pasturing cattle. Singler (1981) reported steelhead (anadromous *S. gairdneri*) growth to be slower in turbid water. Since rainbow trout are obligate visual feeders (Ware 1972), poor water clarity in this dugout may have reduced the ability of the fish to accurately locate food.

In cage studies with high rearing densities, stock density inversely influenced rainbow trout growth prior to any effects on survival. Kilambi et al. (1977) reported growth of cage-reared rainbow trout to be greater at densities of 27.4 and 45.0 kg/m^3 than at 78.2 kg/m^3, but survival was unaffected. Trzebiatowski et al. (1981) demonstrated that percent daily weight-gain values diminished with increasing stocking density (3.3–19.8 kg/m^3) with no effects on survival. Collins (1972) reported no differences in growth and survival for cage-reared rainbow trout stocked at densities ranging from 14.1 to 29.1 kg/m^3, which were likely too low to result in growth reductions.

Feeding Rates

Significant differences ($P \le 0.01$) occurred in both years between food conversions of fish fed the low and high rates (Table 1). Unfed control fish gained little weight in either year, hence the effect of natural food on food conversion and growth of fed fish was assumed negligible.

The optimal daily feeding rate was assumed to be within 3–4% of body weight based on *Wr* and food conversion values (Table 1) and observations of feeding behavior. In 1982, the 4% feeding rate apparently did not contribute to better condition (i.e., *Wr*) than the 2% rate, but did add significantly more ($P \leq 0.01$) weight and length. In 1983, however, the 5% feeding rate did not significantly add length and weight relative to the 3% rate. Tatum (1973) also found no difference in growth and survival between cage-reared rainbow trout fed 3 and 5% of body weight daily.

Differences in feeding behavior appeared to indicate that the optimal feeding rate was between 3 and 4%. Although a floating ration was used, approximately one-fourth of the daily food allotment sank when presented. Fish receiving the low rate in either year immediately fed on both sinking and floating pellets. Those receiving the high rates consumed only sinking pellets initially, but eventually fed on floating feed when observers left the cage area. Virtually all sinking pellets were consumed quickly by rainbow trout, thus eliminating the need to correct for food lost from the cage.

Feeding methods to maximize growth are not currently available for rainbow trout reared in a lentic environment. Cage culturists have used hatchery feeding charts (Whitaker and Martin 1974; Jensen 1979) or have weighed fish at intervals and adjusted amounts of food fed accordingly (Hahn 1974; Kilambi et al. 1977; Trzebiatowski et al. 1981). Fish are either underfed, resulting in lost growth, or overfed, which contributes to higher feed costs. Papst et al. (1982) developed a maximum growth model requiring only temperature and initial fish size for rainbow trout fed at maximum feeding rate. The model can be calibrated for a specific culture system by estimating a constant from past growth records.

Balanced energy budgets have been developed for rainbow trout (From and Rasmussen 1984) and Lake Michigan salmonids (Stewart 1980; Stewart et al. 1983). Once the daily ration can be accurately predicted, time and number of feedings per day should be investigated to further optimize growth.

Stocking Densities

The 1982 stocking density (1.21 kg/m^3; 35 fish/cage) was based on pond work by Halverson et al. (1980). Later in the study it was apparent that a higher density was possible; thus, in 1983 we tested 0.94, 1.40, and 1.88 kg/m^3 (35, 52, and 70 fish/cage, respectively). Differences in temperature profiles and water clarity made the assumption of identical pond conditions invalid. Thus, any statistical interpretation concerning stocking density was spurious.

Maximum rearing densities (kg/m^3) have not been reported in the literature for cage-reared rainbow trout. Rearing densities of 110 (Jensen 1979), 143 (Kilambi et al. 1977), and 170 kg/m^3 (Trzebiatowski et al. 1981) have been tested in various aquatic systems, but were not maximum densities. A maximum rearing density may not manifest itself in reduced survival or final fish production, but rather in reduced individual growth and poor food conversion. Trzebiatowski et al. (1981) reported a proportional increase in final production and food conversion with increasing stocking density (kg/m^3), but found increases in individual weight to be inversely proportional to density. Wedemeyer (1976) suspected that high stocking density hindered movement and feeding, even at maximum or excessive feeding rates. Ivlev (1961) reported a reduction in the amount of food consumed with increasing stocking density with fish other than trout. He suggested that there were probably behavioral effects on feeding due to density.

Water circulation is important since high stocking densities can limit dissolved oxygen. Whitaker and Martin (1974) hypothesized that reduced water circulation was partly responsible for outbreaks of bacterial gill disease and subsequent poor feeding and growth of cage-reared rainbow trout. In bodies of water with poor water circulation such as dugouts, a rectangular cage may prevent low dissolved oxygen problems because these cages have a higher surface-to-volume ratio than other designs and thus more effective water circulation.

Fish Size

The initial size of stocked fish affects the harvest weight, and can be particularly important when the rearing time is brief. A minimum marketable size, considered to be about 170–190 g live weight (Whitaker and Martin 1974; Trzebiatowski et al. 1981), was not achieved from initial stocking sizes of 34.5 and 26.9 g in 1982 and 1983, respectively. Since the rearing time cannot be easily extended, stocking of larger-sized rainbow trout would be necessary. The availability and additional cost of larger fish might limit profits. Whitaker and Martin (1974) found that increasing the initial size of cage-reared rainbow trout from 1.7 to 7.8 or 13.9 g produced marketable fish (173 g live weight) in 122 d. They concluded that, although fish stocked at even 13.9 g will produce a heavier fish at harvest, their higher initial cost was not economically justified. They recommended an initial stocking size of 8 g.

Palatability

Dugout pond owners and others who received fresh rainbow trout from this study responded favorably to the flavor and color but preferred a slightly larger fish. Palatability tests were conducted by Vodehnal (1982) using rainbow trout reared in dugouts. Flavor, odor, color, texture, and overall appearance were favorably rated by a 12-member panel.

Economic Considerations

Rainbow trout readily adapt to the cage environment (Collins 1972) and currently represent the best cold-water species for rearing to a marketable size. Nevertheless careful economic considerations are necessary to receive a favorable return on investment in a culture operation. Cauvin and Thompson (1977) analyzed the economic feasibility of extensive lake culture of rainbow trout in western Canada, but no information was reported concerning the economics of rainbow trout cage culture.

The major economic limitations of a culture operation are the cost of fingerlings and feed, and the low cash return from the marketed product. Necessarily, optimum stocking density and fish size, feeding, cage design, and other factors must be determined for particular rearing conditions to economically achieve maximum growth, production, and survival.

The economics of a hypothetical dugout culture operation were developed for 66-g rainbow trout stocked in 9.82 m^3 (1.22 × 3.66 × 2.20 m deep) rectangular floating cages at 6.6 kg/m^3 (100 fish/m^3; Table 2). An average harvest weight of 190 g live weight was projected for a 60-d rearing period with a 3% daily ration and a 1.65 food conversion. Fingerling fish cost ($35/100) was obtained from a private fish hatchery in St. Paul, Minnesota. A $350 transportation charge ($1.00/mile, one-way) was used for a capacity load (12,400 fingerlings weighing 818 kg). Wholesale prices in 1984 for fresh rainbow trout were $5.03–$5.69/kg. Our estimated production cost ($4.26/kg) resulted in a net profit of $1.43/kg. Estimated cage costs were relatively insignificant to the operation when amortized over 5 years.

TABLE 2.—Estimated cost for a hypothetical 1-year cage-culture operation producing rainbow trout in a 9.82-m^3 (8.93 m^3 available rearing space) rectangular cage in an eastern South Dakota dugout pond.

Item	Cost ($)
Fingerling rainbow trout (66 g each):	
893 fish @ $35/100[a]	313
Fish feed: 183 kg @ $0.88/kg	161
Cage, wood frame with plastic netting[b]	50
Transportation[c]	25
Total production cost	549
Cost/kg trout[d]	4.26

[a] Stocking rate, 100 fish/m^3.

[b] Prorated over 5 years.

[c] Cost based on full-capacity load.

[d] Based on 875 surviving (2% mortality) and 147 g dressed weight (23% live weight loss).

Conclusions

The study demonstrated that cage-reared rainbow trout could successfully grow and survive in eastern South Dakota dugout ponds. Survival indicated that a landowner could expect to harvest a large majority of the fish stocked if adequate care was taken to insure harvest before mortalities from low oxygen and high temperature occurred. The appropriate feeding rate was found to be between 3 and 4% of body weight daily, but an optimal stocking density was not determined. The study showed that a pond owner with a relatively small cage could produce a large weight of acceptably sized rainbow trout in a short time period. The results of a hypothetical cage operation, assuming a daily feeding rate of 3% and a 1.65 feed conversion, indicated that cage culture may be profitable if fish size and stocking density were increased and larger cages used. A cooperative effort among pond owners would minimize fingerling costs and enhance chances of success.

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COMMUNICATIONS

Histochemical Localization of Some Digestive Enzymes in Larval Walleyes[1]

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Abstract. – Although larval walleyes (*Stizostedion vitreum*) readily ingest formulated feeds, survival is low. The present study was conducted to determine if the gut of larval walleyes has digestive capability. Preliminary data indicate that the gut exhibits considerable enzyme activity for four intracellular enzymes – acid phosphatase, alkaline phosphatase, nonspecific esterase, and aminopeptidase – that catalyze the breakdown of a wide variety of organic substances and are capable of digesting many breakdown products of proteins, fats, and carbohydrates. If the digestive system of walleyes becomes functional before the yolk sac is absorbed, as seems likely from the data, problems encountered in feeding formulated feeds to larvae probably do not result from lack of digestive capability.

Despite numerous attempts to intensively culture walleyes (*Stizostedion vitreum vitreum*), larvae have not yet been reared in substantial numbers on formulated feeds. In most hatcheries, larval walleyes are either stocked within a few days after hatching or are maintained on live food. In attempts to develop techniques for complete culture of this important food and sport fish, we are investigating several factors related to feeding and survival of larvae. In studies of feeding (unpublished), we have found that larval walleyes readily ingest formulated feeds, yet survival is low. We undertook the present study to determine if the larval gut has digestive capability. Pertinent to the culture of this species, preliminary data indicate that the gut of larval walleyes is well developed, with considerable enzyme activity, by at least the second day posthatching.

To date, we have analyzed activity of four intracellular enzymes – acid phosphatase, alkaline phosphatase, nonspecific esterase, and aminopeptidase – that catalyze the breakdown of a wide variety of organic substances in cells; monitored as a group, they provide an estimate of the digestive activity of cells. Gut cells showing positive activity for all these enzymes are capable of digesting many breakdown products of proteins, fats, and carbohydrates. Although relatively few specimens have yet been examined, histochemical tests for all four digestive enzymes were so consistently positive that a general pattern is indicated.

Methods

Newly hatched walleyes were obtained from two fish hatcheries (Spirit Lake, Iowa, and Valley City, North Dakota) during April and May 1982. They were maintained in 6-L aquaria at approximately 15°C and fed nauplii of brine shrimp (*Artemia* sp.). To gain an overview of the condition of the larval digestive tract, one or two larvae were killed each day from days 1 to 6 posthatching and then at 2-d intervals until day 30. Whole larvae were quenched in liquid nitrogen and stored at −70°C for later sectioning. Whole, frozen larvae were bound to tissue blocks and longitudinal sections were cut at 8 μm in a cryostat microtome and stored at −20°C before enzyme localization.

Histochemical methods employed for enzyme localizations were: pararosaniline-HCl and sodium nitrate for detection of acid phosphatase (Barka and Anderson 1962); naphthol AS-MX phosphate and diazonium (red violet LB) salt for alkaline phosphatase (Burstone 1958); alpha naphthyl acetate–pararosaniline-HCl for nonspecific esterase (Bancroft 1977); and L-leucyl-β-naphthylamide and KCN for aminopeptidase (Nachlas et al. 1957). In all cases sample sections were incubated in specific substrate-deficient media as negative controls. Two to four replicate slides were prepared for each test.

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Results

Results of tests for all enzymes were positive from day 2 posthatching through day 30. Positive staining reactions for the four enzymes were seen in the ciliated border of epithelial (mucosal) cells of the intestine. In day-1 larvae, tests were positive for nonspecific esterase, weakly positive (staining reaction light compared with that on other days) for acid and alkaline phosphatases, and negative for aminopeptidase. It was difficult to obtain intact frozen sections of the digestive tract of day-1 larvae.

In addition to the positive staining reactions in the gut mucosa, the four enzymes were also detected in other tissues (Table 1). Liver tissue was positive for esterase in a day-1 larva, for acid phosphatase in day-18 larvae, and for alkaline phosphatase in several fish older than 14 d. Kidney tissue was positive for all enzymes: for esterase on days 4, 5, and 24; for acid phosphatase on days 2, 3, 18, and 22; for alkaline phosphatase on days 14, 24, and 26; and for aminopeptidase on day 14. Connective tissue of the head, especially of the eye capsule, also stained strongly for alkaline phosphatase in larvae throughout the sampling period.

TABLE 1.—Results of histochemical tests for four enzymes in intestinal mucosa and other tissues of larval walleyes.

Enzyme and age of larvae (days posthatch)	Number of larvae	Tissue	
		Intestinal mucosa[a]	Other positively stained tissue[b]
Acid phosphatase			
1	2	+–	
2–30	20	+	K, L
Alkaline phosphatase			
1	2	+–	EC
2–30	22	+	K, L, EC, HT
Nonspecific esterase			
1	1	+	K, L
2–30	21	+	K
Aminopeptidase			
1	1	–	
2–30	19	+	K

[a] Staining reactions: + is positive; +– is weakly positive; – is negative.

[b] K = kidney; L = liver; EC = eye capsule; HT = head connective tissues.

Discussion

Considering the positive reactions obtained in gut mucosa, the presence of enzyme activity in other tissues of larval walleyes was not surprising. The phosphatase enzymes cleave inorganic phosphate groups from a variety of molecules, esterases catalyze the breakdown of esters that could be derived from carbohydrates or fat molecules, and aminopeptidases degrade peptides derived from proteins. Vertebrate kidney and liver cells typically have these and other classes of enzymes.

We made no systematic attempt to stain sections containing tissues other than the gut nor to scan all preparations for positive reactions in other tissues. Not all sections contained liver and kidney, and it is likely that many other larvae would have shown positive localizations of enzymes in these tissues if they had been systematically analyzed. Exocrine pancreatic tissue, a major source of digestive enzymes, was not identified in any sections.

Using polyacrylamide-gel electrophoresis, Alliot et al. (1977) recorded activity of eight digestive enzymes, including the four that we monitored, in larvae of the sea bass *Dicentrarchus labrax*. Because extracts of whole sea bass larvae were used, the tissue sources of those enzymes were not identified. However, as in walleyes, activities of all enzymes were weak in newly hatched sea bass and increased progressively with age. Such a pattern may be common among larval perciform fishes. Grizzle and Curd (1978) found that the digestive system of the logperch (*Percina caprodes*) becomes functional before the yolk sac is absorbed. If this proves true for walleyes, as seems likely from our data, problems encountered in feeding formulated feeds to larvae probably do not result from a lack of general digestive capability.

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An Integrated System for Treating Nitrogen Supersaturated Water

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Abstract.—Groundwater is commonly supersaturated with nitrogen and must be treated before it is used for culturing fish—especially sensitive species such as lake trout (*Salvelinus namaycush*) and Atlantic salmon (*Salmo salar*). We treated water with an integrated system that passed water through a packed column aerator, then through a vacuum degasser, and finally through another packed column aerator (installed as a backup system). Packed-column aeration prior to vacuum degassing provided increased efficiency because only a small amount of vacuum was required to remove the remaining excess nitrogen, and oxygen levels were not affected by the vacuum degasser. In well water passed through packed columns, nitrogen gas was reduced from 131 to 105% of saturation and oxygen was increased from 23 to 86% of saturation. With a vacuum pressure of 3 in Hg, the degasser further reduced the nitrogen gas from 105 to 99% of saturation, and oxygen saturation remained near 86%. The integrated system provided water in which all gases were near saturation. No effects of gas supersaturation have been observed among the 18 species of cold-water, coolwater, or warmwater fish that have been cultured in this water.

Nitrogen supersaturation in groundwater is common. In recent years, gas bubble disease (a condition caused by uncompensated pressures of the total dissolved gases) resulting from gas supersaturation has caused the loss of millions of juvenile lake trout (*Salvelinus namaycush*) in hatcheries. Its cause and treatment were reviewed by Rucker (1972), Weitkamp and Katz (1980), and Speece (1981). Safe limits for total dissolved gases in fish culture depend on individual species tolerances, the duration of exposure, barometric pressure, and the temperature and salinity of the water (Bouck 1980). Total gas saturations of only 104–105% have been reported to cause symptoms of the disease in salmonids (Bouck 1980).

Several aeration systems have been used to reduce gas supersaturation. One accepted design is a gravity-flow, packed column consisting of a tube filled with spherical or cylindrical plastic structures that break up the surface tension of the water and release excess gases (Owsley 1981). Packed columns are generally 4 ft or more long and the diameter is selected to accommodate a particular water flow.

Mechanical, vacuum-degassing systems are the only devices capable of reducing gas saturation to less than 100% (T. W. McLaughlin and L. S. Busch, unpublished, 1981). A vacuum-degassing system installed in 1979 at the National Fishery Research Laboratory, La Crosse, Wisconsin, reduced nitrogen saturation from over 130% to near 100%; however, the vacuum pressure of 11 in Hg required to alleviate the nitrogen supersaturation also reduced the water line pressure by about 20 lb/in^2 and further decreased the already low dissolved oxygen concentration from 27 to 22% of saturation (Marking et al. 1983).

In an attempt to overcome the shortcomings of this vacuum degasser, we installed a series of packed columns to aerate the supply water and altered the degasser. The effects of these actions were monitored to determine (1) the efficiency of the packed column aerator; (2) what additional degassing was needed to produce water with nitrogen gas at less than 100% saturation; and (3) whether the integrated system would produce water that was suitable for fish culture.

Equipment Installation and Modification

A series of packed columns that accommodated water flows up to 3,000 gal/min was installed in

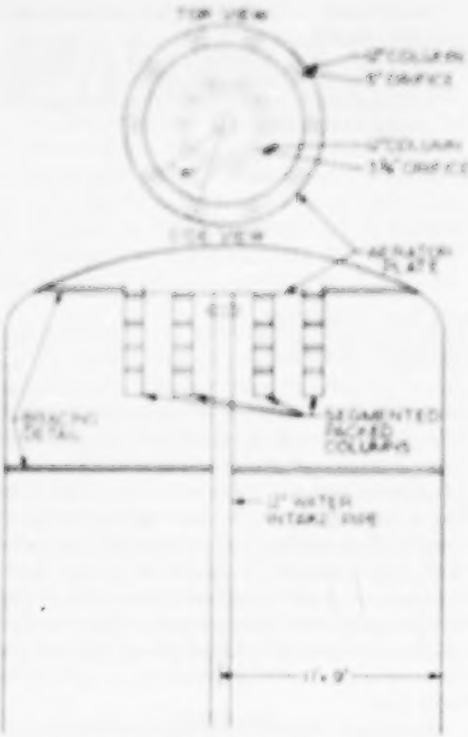
TOP VIEW
12" COLUMN
5" ORIFICE
12" COLUMN
1⅝" ORIFICE
SIDE VIEW
AERATOR PLATE
BRACING DETAIL
SEGMENTED PACKED COLUMNS
12" WATER INTAKE PIPE
17' 9"

Figure 1. – Packed column aeration equipment in water reservoir tower.

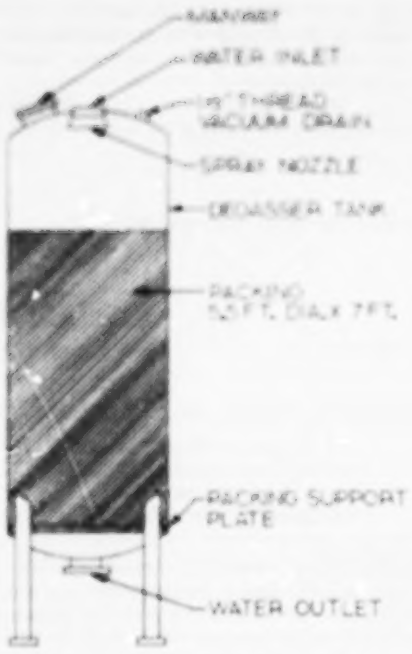
MANWAY
WATER INLET
1½" THREAD VACUUM DRAIN
SPRAY NOZZLE
DEGASSER TANK
PACKING 5.5 FT. DIA. X 7 FT.
PACKING SUPPORT PLATE
WATER OUTLET

Figure 2. – Components and dimensions of vacuum-degassing equipment.

the top of the laboratory's reservoir tower to aerate the well water. This unit was comprised of 20 individual packed columns containing 2-in Jaeger Tri-Packs (spherical plastic structures). Each column contained 4 ft of material in 12-in-diameter segmented sections (Figure 1). The segmented columns were similiar to those described by Owsley (1981) but were modified for greater efficiency.

Water from the packed column aerator in the reservoir tower flowed to the vacuum-degassing system which was modified by placing Tri-Packs in the vacuum tank and replacing the dual 3-horsepower in-line vacuum pumps with two ½-horsepower vacuum pumps and a vacuum storage tank. The Tri-Pack material in the 5½-ft-diameter degassing tank was 7 ft deep (Figure 2). Flow rate through the tank was about 750 gal/min. This modification increased the efficiency and decreased the operational cost of the degassing system. Energy costs for operating the ½-horsepower vacuum pumps were small because the pumps can maintain the required pressure of 3 in Hg by running less than 5% of the time. Water from the vacuum degasser was pumped to packed column aerators, which were installed as backup systems on each fish tank.

Sampling and Measuring Procedures

The aeration system in the reservoir was installed in December 1984, when water flow through the system averaged about 1,000 gal/min. The percent saturation of gases was monitored weekly, during the following 6 months, with an Eco Enterprises Model ES-2 saturometer and a YSI Model 54 ARC oxygen meter. The percent saturation was determined in untreated well water, in water from the reservoir aerator, in water discharged from the vacuum degasser, and in water from the packed column aerators on each fish tank.

Measurements of total gas pressure, dissolved oxygen, temperature, and barometric pressure were used to compute the percent saturation of total gases, oxygen, and nitrogen–argon, according to the methods of Weiss (1970). Computer programs were used to facilitate the computations (Dawson 1986).

TABLE 1.—Mean saturation of gases (SE in parentheses) in untreated well water and after treatment by the reservoir aerator, the vacuum degasser, and the fish tank aerator during a 6-month period.

Water source (number of samples)	Oxygen (ppm)	Saturation (%)		
		All gases	Oxygen	Nitrogen gas
Untreated well water (26)	2.5 (0.16)	108 (0.64)	23 (1.5)	131 (0.52)
Reservoir aerator (27)	9.3 (0.16)	101 (0.08)	86 (1.6)	105 (0.50)
Vacuum degasser (24)	9.3 (0.18)	97 (0.19)	87 (1.2)	99 (0.36)
Fish tank aerator (9)	10.2 (0.15)	100 (0.08)	94 (1.6)	101 (2.80)

Saturation of Gases

Untreated well water averaged 54 ± 2°F and 131% saturation of nitrogen gas during the monitoring period. The packed columns in the reservoir tower reduced the nitrogen and total gases by 26 and 7%, respectively, and increased oxygen levels by 63% (Table 1). The packed columns in the reservoir tower effectively increased the dissolved oxygen concentration to greater than 9 ppm, but they did not reduce the nitrogen concentration to a level that would protect sensitive species and life stages of fish.

The modified vacuum degasser required only 3 in Hg to further decrease the nitrogen and total gas saturation by 6 and 4%, respectively, while producing no reduction in the dissolved oxygen concentration. Water leaving the degasser was not supersaturated with nitrogen, and the vacuum pressure of 3 in Hg had no measurable effect on water pressure in the supply line.

The aerated and degassed water passed through a 4-in-diameter, 5-ft-long packed column and entered each fish-rearing tank at a flow rate of about 7 gal/min. This additional aeration increased the nitrogen, oxygen, and total gas saturation by 2, 7, and 3%, respectively. (The final aeration step was not needed to reduce gas saturation, but it provided a backup for the other systems.) No effects of gas supersaturation have been observed among the 18 species of cold-water, coolwater, or warmwater fish cultured in the treated water. The list of species includes brown trout (*Salmo trutta*), rainbow trout (*Salmo gairdneri*), lake trout, brook trout (*Salvelinus fontinalis*), northern pike (*Esox lucius*), common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), fathead minnow (*Pimephales promelas*), white sucker (*Catostomus commersoni*), channel catfish (*Ictalurus punctatus*), black bullhead (*Ictalurus melas*), striped bass (*Morone saxatilis*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieui*), green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), yellow perch (*Perca flavescens*), and walleye (*Stizostedion vitreum vitreum*).

Gas supersaturation problems are often complex, and usually no single solution is available that meets the economic constraints and biological requirements of fish culture. If the oxygen concentration in the water supply is low, vacuum degassing may not be an acceptable alternative because aeration will be required to replenish the oxygen. If the oxygen content in the water supply is near saturation and nitrogen supersaturation is not excessively high, vacuum degassing alone may be adequate (Fuss 1983). Marking et al. (1983) described an integrated system where the water is first vacuum degassed and then aerated. If aeration and degassing must be integrated, we recommend that aeration be applied first to relieve both supersaturation of nitrogen and undersaturation of oxygen with little cost and effort.

Acknowledgments

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Pattern of Oxygen and Phosphate Uptake by Indian Carp (*Cirrhinus mrigala*) Eggs during Development in the Chinese Hatchery System

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Abstract. – Measurements of oxygen and phosphate uptake rates and liberation rates of carbon dioxide and ammonia by Indian carp eggs were made in the Chinese hatchery system during different hours of development. The uptake rates of oxygen and phosphate tended to rise gradually for 11 h of development and then declined sharply. On the other hand, the liberation rates of carbon dioxide and ammonia sharply increased during hatching and then declined. This suggests that developing embryos become metabolically most active during hatching.

Though a number of hatchery systems are in use in India, few studies have evaluated water quality in these hatcheries. Further, little information exists about water quality associated with development of Indian carp eggs. This information is needed to develop techniques in manipulating water quality for maximum survival of Indian carp spawn in hatcheries. The purpose of the present investigation was to measure the uptake rates of oxygen and phosphate and the liberation rates of carbon dioxide and ammonia by Indian carp eggs during development. This information will be useful in the incubation and hatching of eggs in practical hatchery management, particularly in situations with a limited water supply or in closed water systems.

Methods

The uptake rates of oxygen and phosphate and liberation rates of carbon dioxide and ammonia by developing Indian carp embryos at different stages of development were measured in five Chinese hatchery systems in the Chatterjee Brothers Induced Breeding Fish Farm, Magra, West Bengal, India. The experiments were conducted during the period July through October 1983. Water temperatures at these hatcheries ranged from 27 to 30°C.

The Chinese hatchery system consists of an overhead tank, spawning pool, incubation or hatching tank, and spawn-rearing tank. The circular hatching tank, constructed of brick and cement, consists of two concentric chambers. The chambers have inside diameters of 1.6 and 5.0 m. A water depth of 0.92 m was maintained throughout. The inner chamber is separated from the outer by a fine nylon screen that is stretched and fitted onto an iron frame and sealed with a rubber belt. Circular movement of the water is maintained by several diagonal pipes that direct water to the bottom and sides of the pool; stoppers in the vertical outlet pipes regulate the water flow into the hatching tank.

Adult male and female Indian carp were released in a spawning pool after pituitary injection. Following spawning, fertilized eggs were collected from the spawning pool and transferred into hatcheries that had continuous water circulation. The mean density of eggs in the test hatcheries was $68 \times 10^4/m^3$. Limnological conditions of water in these hatcheries were monitored at regular intervals (Jana et al. 1985).

For determination of chemical uptake and liberation rates, experiments were conducted at each stage of development through 52 h postfertilization. Water quality was monitored in each hatchery prior to egg release. Four 3-L glass jars were filled with hatchery water filtered through 0.45-

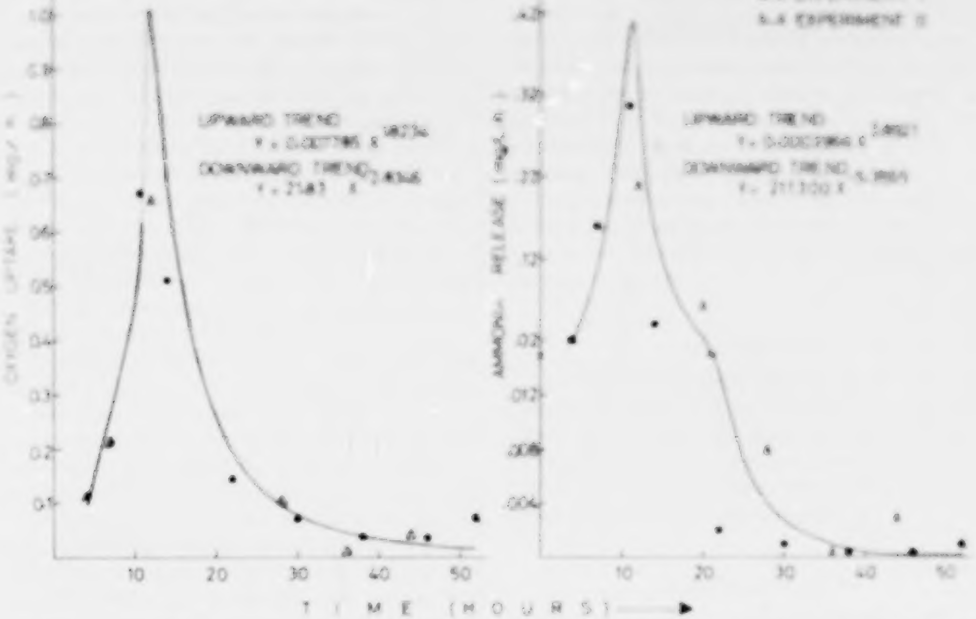

FIGURE 1.—Patterns of oxygen uptake and ammonia release by developing Indian carp embryos during 0–52 h postfertilization.

µm millipore filters to avoid nannoplankton; the jars received developing embryos at different hours of development. The egg mass placed in each glass jar was at the same density as in the Chinese hatchery system. Similar arrangements were made with a control series of four jars that received filtered water but no egg mass. Egg and control jars were then suspended in hatchery water at ambient water temperatures for a specified period of time. The eggs were then removed from the glass jars and the amounts of oxygen, carbon dioxide, phosphate, and ammonia in the water in both series of glass jars were determined. A new series of uptake and liberation measurements was then conducted for the next stage of egg development. The concentrations of oxygen and dissolved ammonia in water were analyzed with specific electrodes (oxygen: model 97-08; carbon dioxide: model 95-02-00; ammonia: model 95-10) in the model 901 Orion microprocessor ion analyzer (Orion Research Institute, Cambridge, Massachusetts 02139, USA). Concentration of ortho-phosphate was determined by the stannous chloride method (APHA et al. 1976). Physicochemical measurements always differed significantly between the experimental and control series. The concentration differences between the initial and final samples of water in the experimental series gave the uptake and liberation rates by the developing embryos. The overall mean rate for each stage was then determined from the four replicate values.

Exponential equations were used to obtain a best-fit curve of the observed rates of oxygen uptake and ammonia excretion. Because the observed curve showed distinctly upward (0–11 h) and downward (12–52 h) trends, two separate equations were used to derive fitted points of the two curves; Spearman's rank correlation test was then performed to find the confidence level of the fitted curve. The level of significance is accepted as being <0.05 or 0.01.

Results and Discussion

Measurements of oxygen uptake and ammonia release by the developing embryos at different hours of development are shown in Figure 1. The uptake rates of oxygen ranged from 0.013 to 1.061 mg/h. The rate of oxygen consumption tended to rise gradually for 11 h of development and then declined sharply. The concentration of oxygen in hatchery waters also decreased as a function of incubation time (Jana et al. 1985). This implied

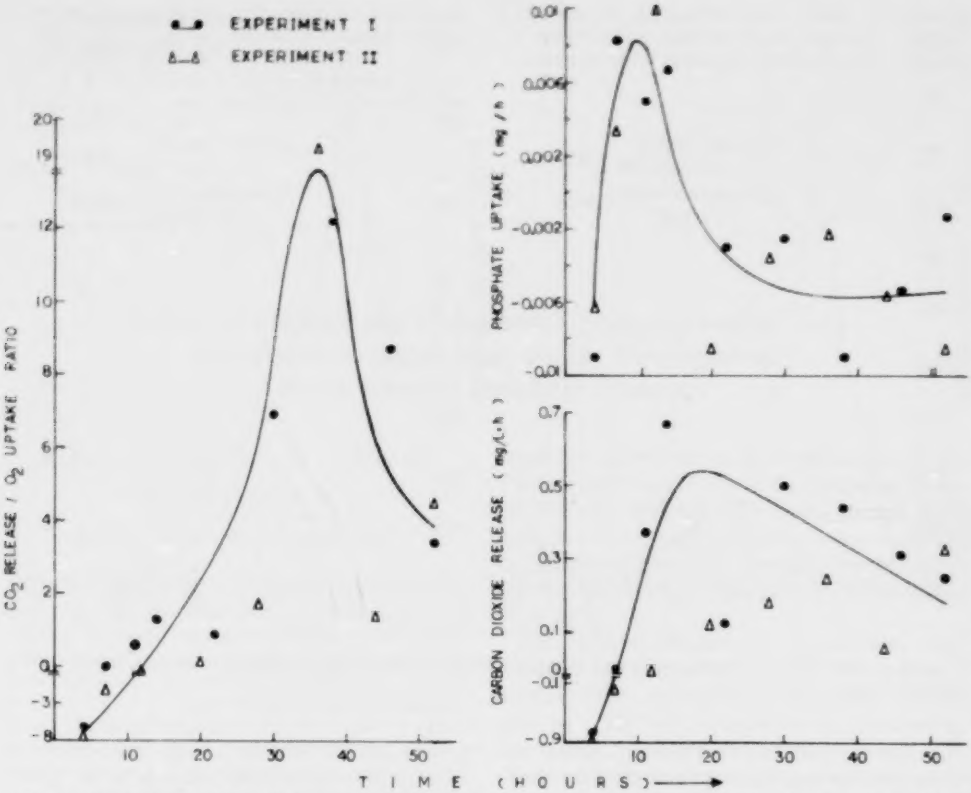

FIGURE 2.—Patterns of phosphate uptake, carbon dioxide release, and the ratio of CO_2 release to O_2 uptake by developing Indian carp embryos during 0–52 h postfertilization.

the differential rate of oxygen uptake during the morphogenesis and larval development of the test fish. The oxygen uptake curve is fitted by two separate exponential equations:

upward trend: $Y = 0.007785X^{1.8234}$; (1)
downward trend: $Y = 21.83X^{-2.8346}$; (2)

Y = oxygen uptake (mg/h) by the developing embryos (6.8 × 10^2/L water);
X = incubation time of developing embryos in hours.

The level of ammonia in the present study was greatly increased (1.8- to 34.78-fold) during the hatching period and then steadily declined (Figure 1). The sharp rise of ammonia level in the water during hatching of Indian carp eggs might be due to the maximal ammonia excretion at this stage. Measurement of ammonia excretion by the fish embryos at different hours of development showed a sharp rise during hatching (first 11 h) followed by a gradual decline (12–52 h postfertilization). Similar to the uptake of oxygen, two separate exponential equations were used to predict the observed rates of ammonia excretion:

upward trend: $Y = 0.0003966X^{2.8921}$; (3)
downward trend: $Y = 211{,}300X^{-5.3555}$; (4)

Y = rate of ammonia excretion (mg/h) by the eggs (6.8 × 10^2/L);
X = incubation time of developing embryos in hours.

Estimates of the ratio of carbon dioxide release to oxygen uptake by the developing embryos (Figure 2) showed a sharp rise after hatching and a decline thereafter. Perhaps due to maximum activity of the developing embryos at hatching, carbon dioxide was liberated from the embryos at a faster rate than the rate of oxygen uptake during the process of hatching, but the reverse occurred for the later period of development. There is evidence that sensitivity to low oxygen tension or anoxia varies with fish species and with stage of development. For example, Blaxter (1969) found that oxygen uptake by the eggs of medaka (*Oryzias latipes*) was very low and there was no sharp rise in uptake until a few hours after fertilization. However, it seems likely that respiratory rate becomes almost unchanged at this stage (Nakano 1981). Further, the oxygen consumption in different stages of northern pike (*Esox lucius*) eggs was found to be strongly affected by water temperature (Nakano 1981). The pattern of oxygen uptake by sea turtle (*Chelonia caretta*) eggs at 30°C appeared to be sigmoidal in nature (Ackerman 1981).

Phosphate levels in hatchery waters declined during hatching (7–14 h); thereafter phosphate levels tended to rise (Jana et al. 1985). The phosphate uptake rates by developing embryos in glass jars (Figure 2) indicate the same trend that occurred in an incubation chamber, with maximum uptake during hatching and a decline thereafter. The developing embryos utilized dissolved phosphate from the water for general metabolism and bone formation, and this uptake was presumably mediated through the permeable cell membrane by a cationic exchange mechanism (Jana et al. 1985) because intake of water through the mouth was not possible at this stage. It is reported that brook trout (*Salvelinus fontinalis*) are able to take up very limited amounts of dissolved phosphorus from water (Phillips et al. 1959).

The changes of water quality coupled with the development of Indian carp eggs were fairly evident. The pattern of oxygen consumption by the developing embryos in different hours of development was very similar to that of phosphate uptake, showing maximum rise during hatching and a decline thereafter. It is further evident that the liberation rates of carbon dioxide and ammonia were much higher during hatching than in the later stages of development.

It is concluded that the results of these experiments may be utilized for maximum survival of Indian carp spawn in hatcheries, especially in situations with a limited water supply or in a closed water system. Because of greatly increased oxygen consumption and production of ammonia and carbon dioxide by embryos during hatching, it is apparent that the developing embryos became metabolically most active during the process of hatching. Therefore, special attention should be given to manipulating water quality to eliminate the harmful effects of excess ammonia and carbon dioxide for maximum survival of Indian carp spawn in hatcheries.

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Evaluation of Thermal and Chemical Marking Techniques for Tilapia

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Abstract.—Hot, freeze (liquid nitrogen), and chemical (silver nitrate and potassium permanganate) brands were applied to juvenile and adult *Tilapia nilotica*. Application efficiency was considered and the persistence and the clarity of marks were evaluated for a 61-d period. For juvenile fish (initial weight 2.2 g), freeze brands were most effective, heat brands were less satisfactory, and chemical brands failed all criteria. No brands, regardless of the application method, persisted when they were applied to adults (mean weight 100 g), or when they were obscured by scale regeneration.

Fish-breeding programs would benefit from the development of suitable multigroup identification systems that would eliminate the need for separate rearing of experimental groups and for large, multiple-pond facilities (Wohlfarth and Hulata 1983). Present marking techniques are limited by the number of identifiable groups, by poor tag retention, and by the difficulty in mark identification.

Numerous noninvasive marking techniques have been developed for fish. These involve thermal or chemical branding of epidermal and subdermal tissue layers. Groves and Novotny (1965) described a heat-branding method with silver-tipped branding irons immersed in boiling water (100°C). Cold branding with an ethanol–dry-ice slurry (−80°C) was found to be slightly superior to heat branding (Fujihara and Nakatani 1967). The cold-branding system was further refined by Mighell (1969) through the use of liquid nitrogen (−196°C) and branding boxes. Successful chemical branding with a silver-nitrate–potassium-nitrate application on channel catfish (*Ictalurus punctatus*) was reported by Thomas (1975).

This study considers the marking of various-sized *Tilapia nilotica* with different thermal and chemical branding methods. Each method was evaluated on the basis of application efficiency and the persistence and clarity of marks in order to determine its suitability.

Heat- and freeze-branding equipment and protocol followed studies by Groves and Novotny (1965) and Mighell (1969), respectively. The technique used for chemical branding differed considerably from that used by Thomas (1975) because we were unable to obtain applicators impregnated with silver and potassium nitrates. Brands produced by chemical "burning" were attempted by applying a 2.0% solution of silver nitrate or a 2.5% solution of potassium permanganate (a less costly oxidizing agent) with a capillary tube 75 mm long and of 1.2-mm inside diameter. Mucus was first wiped off to improve contact with the skin and scales.

Juvenile and adult *Tilapia nilotica* were maintained at the University of Washington and were fed a 40% protein moist hatchery diet (Satia and Brannon 1975). Forty juveniles, 2.2 g average weight, were assigned to each of the four branding groups with each group receiving a distinct letter brand: heat "S," freeze "U," silver nitrate "N," and potassium permanganate "P." All brands were applied dorsal to the lateral line and just posterior to the operculum. Freeze and silver nitrate brands were placed on the right side of the fish, and heat and potassium permanganate brands on the left. Fish were anesthetized in a solution of tricaine (MS-222) prior to branding.

After branding, fish were reared communally in a 220-L circular tank with an inflow of 2 L/min at 28–30°C. Mixing the brand groups provided a simulated field condition.

Twelve adult fish (three per brand group), weighing between 75 and 125 g, were also branded. The brands were placed on three different body locations: the bridge of the snout, the operculum, and posterior to the operculum. The first two locations were selected because of the absence of scales in those areas.

Visual examination determined the number of fish in each group retaining the brand. The brands were also measured to determine the degree of brand growth relative to body growth.

The experiment was conducted for 61 d posthatch during which time the juveniles grew from an average 2.22 ± 0.03 g to 49.79 ± 0.50 g and from 4.84 ± 0.09 cm to 13.24 ± 0.27 cm standard length.

Heat-induced brands were inconsistent in quality, resulting in the misidentification of several fish. Although 40 fish were initially heat branded, 49 were subsequently scored as being heat branded. Twenty-eight of the 49 fish exhibited prominent scars in the branded area. The remaining 21 fish were identified on the basis of abnormal scale patterns resulting in the possible misinterpretation of heat brands for natural scale damage or potassium permanganate brands.

Freeze brands were retained by disruption of the scale pattern. The brands were faint but showed good letter resolution, accounting for the high level (95.0%) of recovery.

The size of the heat and freeze brands increased from an initial height of 0.65 cm to 1.20 ± 0.07 cm and 1.38 ± 0.07, respectively.

Chemical brands produced temporary marks. Only two silver nitrate brands were visible at the termination of the experiment. These were identifiable primarily through the persistence of the cross stroke of the "N" brand. No potassium permanganate brands were found, although some may have been misinterpreted as heat brands. Potassium permanganate brands were not readily visible 3 d after branding, and the majority of the silver nitrate brands were gone in 2 weeks.

Of the 154 fish examined for brand retention, 65 (42.0%) showed no brand marks.

Six juveniles died during the course of the experiment. These deaths occurred sometime after branding and were not a direct result of the branding process itself. No brands were visible on the dead fish.

Two heat- and four freeze-branded juveniles were maintained beyond the initial 61-d period for other experimental purposes. The fish were held in a concrete raceway at 18°C for 7 months postbranding, and they attained a weight of 200 g. Although both brand types were distinguishable, freeze brands were superior in clarity and resolution.

Adult fish were not weighed during the experiment. After 61 d no brands were visible. In adult fish (as in juveniles) freeze and heat brands initially appeared as pigmented scars. The scars in the adults, however, healed within 3 weeks, leaving no trace of the brand. Chemical brands failed to produce any distinct scars. There were no differences in brand retention among the various branding locations.

On salmonids, temperature-induced brands are made visible by the uneven regeneration of scales following branding. Scale pattern disruption may be visible for 2 or more years (Hershberger et al. 1982). The large ctenoid scales of adult tilapia, however, are apparently impervious to temperature- or chemical-induced damage. In scaleless areas, pigmented scar tissue is retained temporarily at best.

The smaller sized and tightly arranged scales of juvenile tilapia are more susceptible to damage and subsequent pattern alteration. Heat branding produced the most pronounced marks after 61 d, but the marks were inconsistent, causing some difficulty in identification. The heat brands also lacked resolution and caused considerable skin damage. Freeze brands provided greater flexibility for multigroup identification. Although faint, the brands were well defined over the 61-d period. The use of fish smaller than 2 g should increase the visibility of the mark by affecting a larger number of scales.

Heat branding may be limited to identifying fish groups by the location of the scar. The number of possible groups identifiable by freeze branding is limited only by the number of distinct letter brands.

The marking of juvenile tilapia with liquid nitrogen proved superior to the other methods. The freeze-branded fish were the only group to exhibit sufficient brand retention on a short-term basis to justify further long-term studies under pond conditions.

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Effect of Initial Feeding Rates of Artemia Nauplii and Dry-Diet Supplements on the Growth and Survival of American Shad Larvae

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Abstract.—An investigation was conducted into the significance of several aspects of diet on the growth and survival of intensively cultured American shad (*Alosa sapidissima*). Artemia (*Artemia salina*) nauplii were fed to larval American shad at rates of 12, 18, or 24 nauplii/fish daily. In addition, two commercially prepared diets (Ziegler AP-100 and Bio-Marine Artemia Flakes) were offered as a food supplement to larval fish that received 12 artemia nauplii/fish daily. The feeding rate of 12 nauplii/fish resulted in the lowest growth and survival. Feeding rates of 18 and 24 nauplii had mixed results with insignificant differences in growth and survival. Dry prepared diets (particularly diet AP-100) offered in combination with artemia nauplii enhanced larval fish survival. First-feeding larval American shad (10.4 mm long) accepted the prepared feed and it remained a major portion of the diet. Particle size appeared to have a major influence on acceptance of diets by American shad larvae.

During the transition from endogenous to exogenous nutrition, cultured American shad (*Alosa sapidissima*) experience a period of low feeding accompanied by slow growth and high mortality related to food deprivation (Wiggins et al. 1985). Adequate feeding regimes must be established for intensively cultured American shad larvae during this period.

Artemia nauplii (*Artemia* spp.) are an essential component of the diet for intensive culture of larval American shad. Although evidence has been presented that some early losses of cultured American shad larvae are due to starvation, there has been no determination of optimum quantities of artemia nauplii that should be fed to these fish. It is surmised that an optimum availability of food would enhance survival of cultured American shad.

The presence of adequate densities of suitable prey organisms is of crucial importance to the survival of larval fishes (Werner and Blaxter 1980). It has been shown that the number of striped bass surviving to stockable size is directly related to the quantity of artemia cysts incubated to feed the larvae (Lewis and Heidinger 1981). The quantities of artemia nauplii necessary to feed larval fish vary considerably with the species. Using rate of digestion, Rosenthal and Hempel (1970) estimated that early postlarval Atlantic herring (*Clupea harengus*) could digest 35–40 artemia nauplii daily. Experimentally, Werner and Blaxter (1980) determined threshold prey densities for larval Atlantic herring to be approximately 0.17 artemia/mL. In contrast to this low prey density, it has been estimated that striped bass larvae initially must be fed at a daily rate of 50–60 nauplii/mL rearing tank volume until larvae are 15 d old. After 15 d, the density of nauplii is increased to 100–120/mL daily (Lewis and Heidinger 1981).

The objectives of this study were to determine the threshold feeding rates for American shad larvae raised on artemia nauplii and the effects on survival and growth of dry prepared diets offered as a food supplement.

Methods

Tests were conducted in 1,200-L, conical, fiberglass rearing units that had a diameter of 1.52 m at the top and 1.37 m at the bottom and a depth of 0.76 m.

Fertilized American shad eggs were obtained from ripe fish captured in a commercial gill-netting operation and were incubated in 6.5-L, plastic egg-incubation jars in the manner described by Wiggins et al. (1984, 1985). Estimates of the number of larvae hatched into each rearing unit were obtained using a modification of von Bayer's (1910) method of egg enumeration to determine the total number of eggs and the number of dead eggs.

Larval American shad were initially stocked at a mean density of 208 fish/L (250,000 fish/tank) with fish densities ranging from 158 to 270 fish/L. Age-specific survival rates through the first 18 d were determined for each test unit as described by Wiggins et al. (1985). Because hatch occurred over a 3–6-d period, a single hatch date was assigned to each lot on the day the majority of fry hatched (generally the seventh day of incubation at 15.6°C). Feed was first presented to larvae at 4 d posthatch.

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Commercial diets were dispensed from a single vibrator-type feeder (model AF6, Sweeney Enterprises). All rearing units received suspensions of newly hatched *Artemia salina* nauplii after nauplii were separated from unhatched cysts (Sanders Brine Shrimp Company). Nauplii were released from two live-food dispensers (a modification of a dispenser developed by Theis and Howey [1981]) during daylight hours (8–12 h daily). Feeding was for 5 s every 5-min period. The volume or mass of feed was based on the initial density of fish stocked in a rearing unit, the feeding rate that unit was to receive, and the density of the newly hatched nauplii.

Three feeding rates were compared – 12, 18, and 24 artemia nauplii/fish daily – based on the initial stocked densities of fish in a tank. Feeding rates were chosen to bracket the feeding rate of 18 artemia/fish used in 1984 at the Pennsylvania Fish Commission's Van Dyke Research Station for Anadromous Fishes, and to include the feeding rate of 12 artemia/fish used in 1983 (Wiggins et al. 1985).

Three rations were compared: (1) 12 artemia nauplii/fish fed in combination with milled artemia flakes (12A + AF); (2) 12 artemia nauplii/fish fed in combination with prepared dry diet AP-100 (12A + AP-100); and (3) 18 artemia nauplii/fish (18A). (Artemia flakes came from Bio-Marine, the prepared diet was from Zigler Brothers.) The dry prepared diets were to replace one-third of the artemia nauplii in the diet. The quantity of dry prepared diets fed, therefore, was a mass equal to twice that of the mass of six live artemia nauplii per fish (the manufacturer recommended the mass of AP-100 diet be equivalent to twice the mass of live artemia nauplii). The mass of live artemia was determined to be 46,000 nauplii/g.

The milled artemia flakes were provided in four sizes: 150 μm, 220 μm, 400 μm, and 800 μm. Diet AP-100 was available in five sizes, but only the smallest sizes (150, 200, and 250 μm) were used in the study. Comparable feeding regimens were established for the different particle sizes during the study.

Twenty-five fish, from 4–8 d of age, were sampled at 2-d intervals for incidence of feeding and growth. Samples were taken 4–6 h after initial feeding. Percent incidence of feeding (IF) was determined by

$$IF = \frac{L_i}{N_i}(100);$$

N_i = total number of larvae sampled on day (i);

L_i = number of larvae with feed (dry feed, artemia nauplii, or both) in the gastrointestinal tract.

The number of nauplii in the gastrointestinal tract of feeding larvae was also recorded.

Each treatment was run in triplicate. Analysis of variance (Snedecor 1957) was computed for growth with $P < 0.05$ accepted as the minimum level for significance.

Results and Discussion

There was no significant difference in length increase of larvae among the three feeding rates (Table 1). Larvae receiving feed at daily rates of 12 or 18 artemia/fish grew a mean of 0.8 mm in length compared to 1.2 mm for larvae fed 24 artemia/fish. The mean numbers of artemia nauplii found in the gastrointestinal tracts of fish fed at the rates of 12, 18, and 24 nauplii/fish were 2.8, 2.9, and 3.3, respectively, which was consistent with differences in growth. Survival was lowest (65.9%) for larvae receiving 12 artemia/fish, highest (77.8%) at the feeding rate of 18 artemia/fish, and intermediate (74.5%) at the rate of 24 artemia/fish. Feeding intensity, measured as the incidence of feeding, paralleled survival, with the highest incidence of feeding (82%) generally occurring in fish receiving 18 artemia/fish, lowest (72%) in fish receiving 12 artemia/fish, and intermediate (81%) for fish receiving 24 artemia/fish.

The study demonstrated that a daily feeding rate of 12 artemia nauplii/fish did not produce fish as well as higher feeding rates. The difference in

TABLE 1. – Mean growth, survival, and number of artemia nauplii found in the gastrointestinal tract of American shad larvae at 18 d of age at initial daily feeding rates of 12, 18, and 24 artemia nauplii/fish.

Daily feeding rate (artemia/fish)	Length increase (mm)	Survival (%)	Number of artemia in gut
12	0.8	65.9	2.8
18	0.8	77.8	2.9
24	1.2	74.5	3.3

growth between the two higher feeding rates was insignificant, the data suggest that a daily rate of 18 artemia/fish (highest survival) was the optimum feeding rate tested.

Dry prepared diets were first presented to 4-d-old larvae. First feeding larval American shad (mean total length 10.4 mm) accepted dry diets (150 µm), and incidence of feeding on the first day was 5% for fish receiving artemia flakes and 19% for fish receiving the AP-100 (Table 2). The highest incidence of feeding on artemia flakes (33%) was noted for 8-d-old larvae. The incidence of feeding on AP-100 diet continued to increase until 10 d of age when 80% of the fish had fed on this formulated diet. There was an apparent decline in the incidence of feeding on AP-100 after day 12, which coincided with a shift to the largest particle size (250 µm).

There was no apparent difference in the initial incidence of feeding when comparing the average of the two feeding rations supplemented with dry diet, 12A + AF and 12A + AP-100 (21%) to the 18A control diet (25%). Subsequent increases in the incidence of feeding through day 12 were similar for the 12A + AP-100 and the 18A regimes. Throughout the study, the incidence of feeding was lowest for the 12A + AF diet (Table 3).

There were no significant differences in length increase among diets; however, the greatest increase (1.6 mm) was demonstrated in the 12A + AP-100 feeding regime. Increases in length of larvae receiving diets 18A and 12A + AF were 1.1 mm and 0.9 mm, respectively.

The lowest mean survival was obtained in fish receiving the 18A diet (47%). Fish fed diets 12A + AF and 12A + AP-100 appeared to have much higher survival rates (66 and 84%, respectively) than those fed diet 18A (Figure 1).

TABLE 2.—Mean incidence of feeding for American shad larvae (day 4 through day 16 posthatch) offered a daily diet of 12 artemia nauplii/fish supplemented with artemia flakes or with formulated AP-100.

Larva age (d)	Incidence of feeding (%) on diets of: 12 artemia + artemia flakes	12 artemia + AP-100
4	5	19
6	24	52
8	33	65
10	27	80
12	15	80
14	24	51
16	12	45

TABLE 3.—Mean incidence of feeding for American shad larvae (day 4 through day 16 posthatch) offered daily rations of 18 artemia nauplii/fish or 12 artemia nauplii/fish supplemented with artemia flakes or with formulated diet AP-100.

Larva age (d)	Incidence of feeding (%) on diets of: 18 artemia	12 artemia + artemia flakes	12 artemia + AP-100
4	25	11	31
6	63	67	81
8	79	60	79
10	76	69	73
12	93	53	86
14	97	61	81
16	89	61	69

Artemia nauplii continue to be an essential component of the diet for larval American shad. These studies, however, demonstrated that dry prepared feeds were accepted by first-feeding American shad larvae and that these feeds remained a major portion of the diet. The diets greatly reduced the high larval mortality experienced in previous studies (Wiggins et al. 1985).

It was apparent that dry prepared feeds (particularly the formulated AP-100 diet) offered in combination with artemia nauplii enhanced survival, although the reasons for this were not clear. The

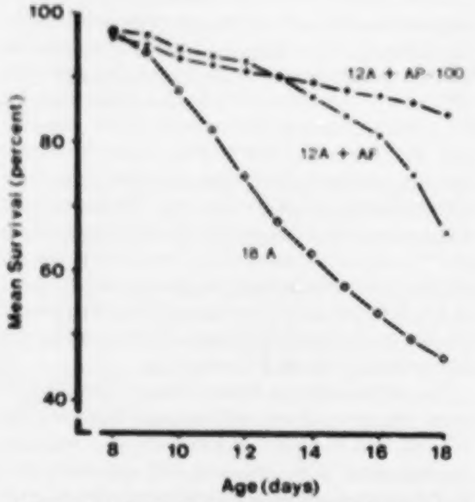

FIGURE 1.—Survival of American shad larvae from age 8 to 18 d when larvae were presented with daily rations of 18 artemia nauplii/fish (18A), 12 artemia nauplii/fish + dry milled artemia flakes (12A + AF), or 12 artemia nauplii/fish + dry formulated diet AP-100 (12A + AP-100).

quantities of feed could not be adequately compared; however, feed particle size may have affected survival rates.

Fish fed artemia nauplii supplemented with dry prepared diets received a larger quantity of feed than fish fed nauplii alone. The moisture content of prepared feeds would be less than that of artemia nauplii which would, in effect, increase the quantity of prepared feed available to the larvae. However, in the previous test there was no apparent difference in larval survival for daily feeding rates of 18 and 24 nauplii/fish. Although artemia nauplii are presently an essential component of the diet for larval American shad, increasing the quantity of nauplii does not yield the increased survival that was obtained when dry prepared diets were used with artemia nauplii.

Initially, the particle size of the prepared diets was smaller than artemia nauplii. Average width of nauplii without appendages is 0.19 mm (Wiggins et al. 1985), very similar to the 200-μm particle size of the dry prepared diets. Through 8 d of fish age, particle size for both prepared diets was 150 μm and there was little size difference through 10 d of age (200 μm for AP-100 and 220 μm for artemia flakes). After 10 d of age, 400- and 800-μm flaked artemia and 250-μm AP-100 diets were fed. Particle size may have influenced the incidence of feeding because it declined sharply (from 80 to 51%) with a change in particle size from 200 to 250 μm; incidence of feeding for AP-100 was much higher than for artemia flakes throughout the study.

A higher mortality occurred after 14 d of age among larvae receiving artemia flakes than among those receiving the smaller size AP-100 diet. Differences in survival between the dry diets may have been partially due to differences in particle size later in the test. This would explain the low initial mortality for larvae fed both supplemental diets, and the delayed mortality which occurred in groups receiving artemia flakes.

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Tissue Chloride Levels of Coho Salmon Fry during Progressive Salinity Exposure

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Abstract.—Coho salmon fry were exposed to progressive increases in salinity of tank water starting at 0% seawater and increasing weekly to 9, 18, and 42% seawater. Control fish were maintained at 0% seawater for the 3-week test period. Tissue chloride levels were measured after 35-min and after 7-d exposure to the test salinity. Tissue chloride levels in fish exposed to 18 and 42% seawater for 35 min rose significantly over that of the control fish. After 7-d exposure, only fish exposed to 42% seawater were unable to maintain chloride tissue levels similar to those of control fish. Measurement of tissue chloride level appears to be a sensitive method for assessing the osmoregulatory ability of small salmonids. The procedure is rapid and simple and can be used on individual fish.

Determination of seawater tolerance has become a routine monitoring procedure in efforts to optimize survival of salmonids in both net-pen culture and hatchery smolt production (Wedemeyer et al. 1980). Methods for determining the seawater tolerance of salmonids have centered on measuring plasma or serum ion balance in fish exposed to seawater. The ability to maintain ion balance is indicative of hyperosmotic regulatory ability and salinity tolerance (Clarke and Blackburn 1977; McCormick and Naiman 1984; Miwa and Inui 1985; Barron 1986). Earlier studies demonstrated that tissue ion balance was also very sensitive to osmotic stress (Black 1950; Parry 1961) and that tissue ion levels may be more sensitive to external salinity than serum or plasma levels and more convenient to measure in small fish. The objective of this study was to determine the effect of progressive increases in salinity (0, 9, 18, 42% seawater) and length of exposure (35 min or 7 d) on tissue chloride levels in coho salmon (*Oncorhynchus kisutch*) fry. A procedure for monitoring tissue chloride levels of small salmonids is presented and the utility of this methodology is discussed relative to current methods of assessing seawater tolerance.

Methods

Coho salmon fry (2–5 g) were obtained from Smith Farm, Corvallis, Oregon, and randomly assigned to test units. The test units consisted of one 19-L control aquarium with 10 fish and two 38-L experimental aquaria with 22 fish each. The units were equipped with airstones and a cotton-charcoal filter and were maintained at 11–12°C. Fish were acclimated to dechlorinated tap water (mean salinity 0.06 meq Cl^-/L) for 7 d prior to initiation of the salinity treatment. Fish were fed commercial trout pellets at about 2% of their body weight once per day. The pH of control and experimental aquaria was measured weekly and remained relatively constant (Table 1). The salinity in the two experimental aquaria was adjusted once every 7 d for 3 weeks (April 23–May 7, 1980) by static renewal with filtered seawater (Newport, Oregon; mean salinity of 31‰; 444 meq Cl^-/L). Control aquarium water was maintained by static renewal with dechlorinated tap water.

Two fish from each experimental aquarium were sampled for chloride analysis 35 min and 7 d after each adjustment of seawater concentration. Salinities progressed from 0% seawater (SW) to 9, 18, and 42% SW. Water samples from all three aquaria were taken for chloride analysis at the time of fish sampling. Chloride levels of the control fish were measured at the beginning and end of the experiment for comparison with fish from treatment tanks and to identify possible temporal changes in tissue chloride levels.

Fish were eviscerated and the head and branchial cavity removed; they were blotted dry and weighed to the nearest 0.01 g. Each fish was then homogenized in 10% perchloric acid to precipitate large molecular weight compounds. The homogenate was centrifuged in a microcentrifuge and a sample of the supernatant was removed for chloride analysis. Chloride analysis of fish and water samples was done with an Amino-Cotlove Chloride Titrator. Chloride tissue concentrations were recorded as meq Cl^- per liter per gram of eviscerated fish. Comparison of means was done by Student's *t*-test. Data for fish from replicate salinity treatments were combined for analysis because

TABLE 1.—Mean tissue chloride levels of coho salmon fry exposed to increasing levels of salinity; N = 6 at each sample time.

Experimental period	Water salinity		pH	Mean (SE) tissue chloride (meq Cl⁻/L g) after	
	meq Cl⁻/L	% seawater		35-min exposure	7-d exposure
Start	0.06	<0.1	7.2	13.86 (0.88)[a]	
Week 1	38.56	8.6	6.9	14.52 (1.03)	15.06 (2.30)
Week 2	81.82	18.4	7.2	19.29 (1.56)	14.89 (1.56)
Week 3	188.33	42.4	7.3	18.57 (1.63)	32.97 (2.47)

[a] Mean tissue chloride of control fish sampled at initiation and end of experiment (N = 12).

analyses of variance indicated no significant differences between experimental tanks ($P > 0.10$).

Results

Mean (±SE) tissue chloride levels of control fish were 14.73 ± 1.90 meq Cl⁻/L at the beginning of the experiment and 12.98 ± 0.95 3 weeks later. These values were not significantly different so any changes in tissue chloride levels of test fish could be attributed to the salinity treatment. No mortality of control or experimental fish occurred during the course of the experiment.

Fish exposed to 8.6% SW for 35 min maintained osmoregulatory homeostasis, but at 18.4 and 42.4% SW, tissue chloride levels rose significantly after 35 min (Table 1). Presmolt coho salmon exposed to 8% SW are nearly isosmotic to the medium and do not normally show any rise in body chloride levels (Black 1950). However, at moderate salinities (10–30% SW), coho salmon show initial rises in blood and tissue chloride levels that generally subside after several hours (Parry 1961; Miles and Smith 1968). In the present study, no significant elevations in tissue Cl⁻ levels were found after 7-d exposure to 8.6 and 18.4% SW, but fish exposed to 42.4% SW were unable to maintain tissue chloride levels relative to control fish.

Discussion

Although prior salinity exposure may influence later osmoregularity ability, previous studies with coho salmon parr found substantially elevated tissue chloride levels at hyperosmotic salinities (Black 1950). The present study showed that coho salmon fry exposed to increasing salinity also regulate tissue chloride ion balance in relation to salinity and time of exposure. Brook trout (*Salvelinus fontinalis*) show a similar response to increasing salinity (McCormick and Naiman 1984).

Measurement of tissue chloride levels is a rapid procedure, requires a minimum of equipment and technical expertise, and can be used on individual fish. Determination of serum or plasma ion balance requires intricate analytical techniques and may involve large experimental error due to contamination from interstitial fluids. Pooling of samples is often necessary or larger fish must be used because a 5-g salmonid has less than 200 µL of plasma (assuming 50 mL/kg blood volume and 28% hematocrit: Smith 1966; M. Barron and coworkers, unpublished). Further, tissue chloride levels appear to be more sensitive to changes in external salinity because chloride content of muscle tissue and tissue hydration can vary considerably, but plasma levels are closely maintained by the fish (Eddy and Bath 1979; Eddy 1982). The greater plasticity of tissue chloride levels relative to blood is evidenced by the 140% increase in the present study, whereas plasma or serum osmolarity and Na concentration generally show maximum increases of less than 100%, and typically deviate less than 50% from control levels (e.g., Eddy and Bath 1979; Miwa and Inui 1985). Determination of tissue chloride levels appears to be a sensitive method for assessing the osmoregulatory ability of small salmonids where blood sampling is inappropriate. Measurement of tissue chloride levels may prove useful in routine monitoring of seawater tolerance of small salmonids during seawater transfer in intensive cultivation units or in smoltification assessments.

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Mercury Content of Shrimp (*Penaeus vannamei*) Reared in a Wastewater-Seawater Aquacultural System

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Abstract.—*Penaeus vannamei* were reared in two ponds, one receiving 19% wastewater in seawater and no feed, and the other receiving only seawater and a prepared commercial feed. The pond receiving the wastewater had significantly more mercury in the sediment, yet shrimp in this pond did not accumulate significant amounts of the mercury in their edible tissue.

One of the most promising directions that aquaculture has taken recently concerns the use of treated wastewater which, when added to large culture ponds, will stimulate production and partially or completely eliminate the need for supplemental feeding (Ryther et al. 1972). However, little work has been done on the uptake of contaminants by organisms in such culture systems. The purpose of this project was to culture shrimp in wastewater–seawater ponds and observe the accumulation of mercury in the edible and nonedible portions of the shrimp. Production and growth data were presented by Landau (1983).

Methods

Two earthen ponds (10 × 10 × 1 m) were lined with polyvinyl chloride and plumbed with a flow-through water system (4-d turnover time) delivering seawater (16–38‰) from the Harbor Branch Foundation ship canal, an extension of the Indian River estuarine system in eastern Florida.

Initially, both ponds received only seawater. In May each pond was stocked with 2,400 postlarval shrimp (*Penaeus vannamei*) and fertilized for 4 weeks with sodium nitrate and sodium phosphate (10 mg N/L and 1 mg P/L, respectively). After this 6-week stabilization period, pond A received a prepared feed, Ralston Purina Experimental Ration 1.F (Ralston Purina, St. Louis, Missouri), and pond B received only seawater plus treated wastewater (9:1, seawater : wastewater) from the laboratory's secondary (activated sludge) sewage treatment plant.

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Samples were collected from each pond every 6–8 weeks for analysis. Ten to 20 shrimp were captured in traps during each collection; they were dissected, placed in a plastic bag and stored frozen (−20°C). The edible muscle from the tail and the nonedible tissue consisting of the head, gut, and carapace were analyzed separately. Sediment from the ponds was collected in glass jars and stored on ice for several hours; the supernatant then was decanted off and the sediment frozen. Pond water and wastewater were collected in 1-gal glass jugs, filtered through nitric-acid-washed glass fiber filters, and stored in HF-washed pyrex bottles for analysis.

All sediment and shrimp samples were freeze-dried then dissolved at 40°C with redistilled nitric acid and hydrogen peroxide in closed flasks to avoid loss of mercury vapor. Care was taken, according to procedures described by Tuffy and Presley (1982), to avoid contamination. The mercury content was measured with a Mercury Monitor, model 1235 (Laboratory Data Control, Riviera Beach, Florida).

Results

The concentration of mercury in the edible tissue (Figure 1) was initially slightly higher in the shrimp from pond A than from pond B (0.30 and 0.23 μg/g dry weight, respectively); this difference was not significant ($P > 0.05$, Wilcoxon test; Scheffler 1980), nor was there any difference throughout the rest of the experiment ($P > 0.05$, sign test for matched pairs; Scheffler 1980). The mercury content decreased from July through October and then leveled off at approximately 0.1 μg/g for shrimp in both ponds.

The nonedible tissue contained more mercury than the edible tissue (Figures 1, 2). There was no difference between shrimp from ponds A and B

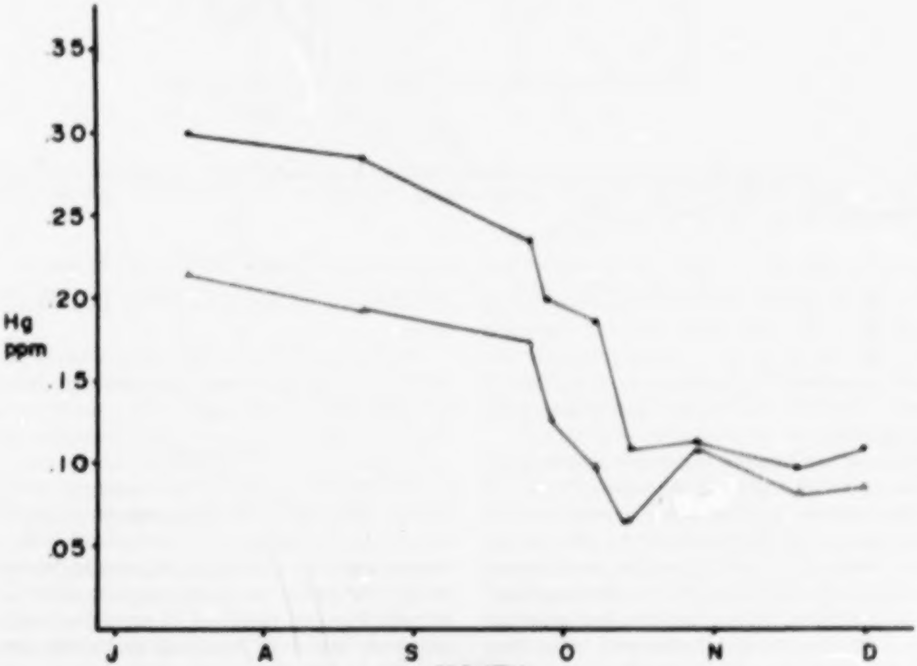

FIGURE 1.—Mercury content in the edible portion of shrimp reared in the control pond (solid dots, upper curve) and in the pond receiving wastewater (open triangles, lower curve). Concentrations represent the average of 10–20 replicate samples expressed as μg Hg/g freeze-dried tissue (ppm).

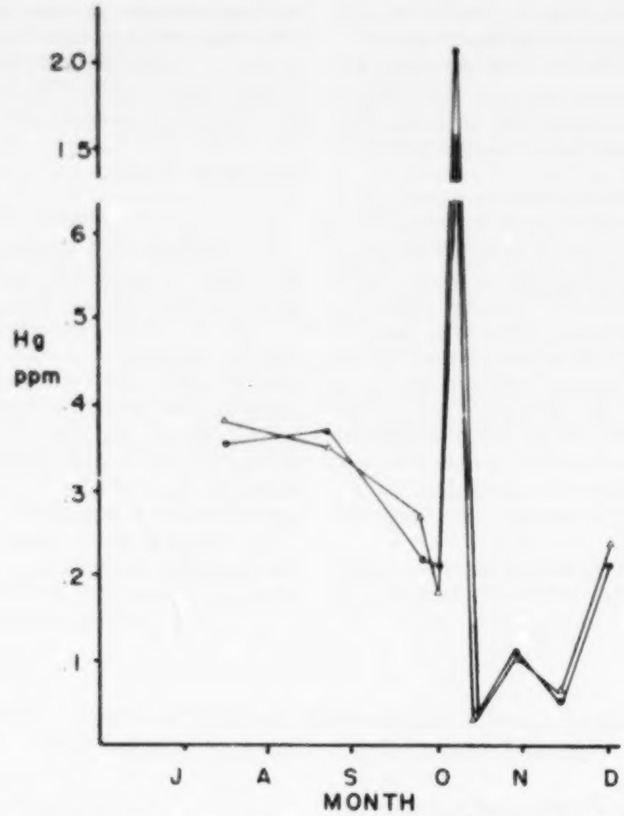

FIGURE 2.—Mercury content in the nonedible portion of shrimp reared in the control pond (solid dots) and in the pond receiving wastewater (open triangles). Concentrations represent the average of 10–20 replicate samples expressed as μg Hg/g freeze-dried tissue (ppm).

($P > 0.05$; Figure 2). The concentration in young shrimp was 0.35–0.39 μg/g in July and decreased to 0.22 μg/g in October. A mercury increase was observed in nonedible shrimp tissue in both ponds in early October, but the levels fell again very quickly.

Sediment mercury content was higher in pond B than in pond A ($P < 0.05$) during the first 4 months of the study (Figure 3). The mercury concentrations in the sediments at the start of the experiment were 20 and 40 μg/g in ponds A and B, respectively. The concentration decreased rapidly in pond A by September, then fluctuated between 1 and 6 μg Hg/g with one peak in late September and another in mid-November. Pond B exhibited a reduction in sediment mercury content from July to August, a rapid increase in late September (as did pond A), a decrease in October, then fluctuations from 0.5 to 10 μg/g through December.

The concentration of dissolved mercury in pond water and wastewater was below the instrument's detection limit (<0.01 μg/L).

Discussion

Mercury was present in the sediment of both ponds, reflecting background concentrations. The source of this background contamination is not known, and no studies of the mercury in the water of the Indian River estuary system were performed. Possible points of origin are municipal sewage outfall, fallout from fossil-fuel power plants, agricultural runoff, or waste effluent from a large local manufacturer of electronic components. Since pond B exhibited higher levels of mercury than pond A during the first 4 months, mercury in the

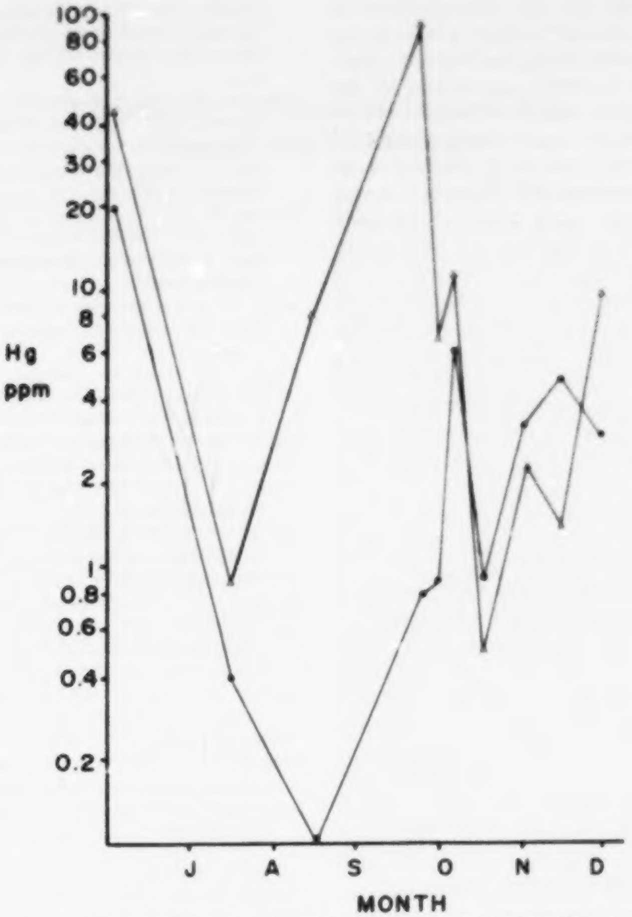

FIGURE 3.—Mercury content of pond sediment collected from the control pond (solid dots) and the pond receiving wastewater (open triangles). Concentrations are expressed as µg Hg/g freeze-dried sediment (ppm).

laboratory's wastewater system is suspected; Klein and Goldberg (1970) found high levels of mercury in the sediment near sewer outfalls. Shrimp raised in pond B did not contain more mercury than those from pond A, indicating that shrimp did not accumulate significant amounts of mercury from the sediment.

Some general trends in mercury content were evident, such as the high levels in the edible tissue at the beginning of the experiment which decreased over time (Figure 1). The decrease in the concentration of certain metals with age (size) is a phenomenon observed in the tissues of some marine organisms (Boyden 1974). The final mercury concentrations in the edible tissues were similar to those reported for several other species of *Penaeus* (Sidwell 1981).

Mercury concentrations in the lipid-rich nonedible tissues were greater than those in the edible tissues. Gilmartin and Revelante (1974) found that fish accumulate more mercury in the liver and kidney than in the muscle. The concentration of mercury in lipid-rich tissue was explained by Simkiss (1983), who suggested that the uncharged, inorganic mercury complexes formed in saline solutions are fat-soluble, and may be 10^6 times as mobile across lipid membranes as other ions such as K^+ or Na^+. Also, when mercury is metabolized to methyl- or dimethylmercury, it becomes more lipophilic.

Oscillations of the mercury concentrations in the sediments may be due to either variable inputs of mercury to the ponds or fluctuations in the mercury-metabolizing bacterial populations in the pond (Wood et al. 1968; Jensen and Jernelov 1969) which produce volatile organic forms of mercury that can escape to the atmosphere. Mercury levels in the sediments were very high, especially during September. Although much greater levels have been reported from atypical sources, such as Minamata Bay, Japan (Takeuchi 1972), most values obtained for sediments are considerably lower (Klein and Goldberg 1970; Cranston and Buckley 1972; Van Faassen 1975; Rae and Aston 1981). However, the detritus within our ponds was mostly biogenic, consisting largely of algae and cyanobacteria; Funk et al. (1973) concluded that algae were prime concentrators of metals, including mercury. Hannan et al. (1973) grew *Chaetoceros* in media containing mercury and found concentrations in those cells of up to 7,400 μg/g. Therefore, mercury in the sediments of our ponds may be associated with benthic algae which, perhaps, were not readily assimilated by the shrimp.

We have shown that shrimp cultured in ponds with chronically elevated levels of mercury in the sediment did not exhibit mercury concentrations in the edible tissues above those found in shrimp collected from natural marine environments. The fact that there seemed to be no appreciable bioconcentration of mercury in the edible tissue is encouraging to culturists who might wish to replace or supplement costly commercial diets with treated wastewater as a nutrient source.

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Potential for Nitrosamine Formation in Seven Fishery Chemicals

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Abstract. – In recent years, nitrosamines have been reported as possible causes of cancer, mutations, or birth defects. Inasmuch as these compounds may be formed by the interaction of certain amines with nitrite in the aquatic environment, we evaluated seven fishery chemicals for their potential to form nitrosamines: the experimental fish toxicant digeranylethanolamine (GD-174); the four therapeutants Terramycin, erythromycin, Hyamine 1622, and Hyamine 3500; and the two tracer dyes rhodamine B and rhodamine WT. The results indicate that the controlled use of the seven fishery chemicals in natural environments will not lead to the formation of nitrosamines.

A wide range of nitrosamine structures, several of which occur in foodstuffs (Crosby and Sawyer 1976), have been reported to cause cancer, mutations, or birth defects (Olajos 1977). Reactions between nitrite and certain amine compounds in an aquatic environment might be expected to result in the formation of nitrosamine, and Meyers and Hendricks (1982) reported that several nitrosamines caused cancer in fish. As part of a Congressional mandate, the U.S. Environmental Protection Agency (1977) required that all pesticides – including fishery chemicals – be tested for their potential to form nitrosamines by interaction with nitrite.

The objective of the present work was to determine if nitrosamines were formed either in water or in fish treated with certain chemicals used in fish culture or management. The chemicals selected were the experimental carp toxicant digeranylethanolamine (GD-174); the four therapeutants Terramycin, erythromycin, Hyamine 1622, and Hyamine 3500; and the two tracer dyes rhodamine B and rhodamine WT. Several other fishery chemicals were excluded from this study because their molecular structure did not contain the nitrogen group involved in the formation of nitrosamines.

Methods

All reagents and solvents used were analytical grade. Inorganic and organic salts were obtained from Alpha Products, Danvers, Massachusetts; sodium alkanesulfonate from Eastman Kodak, Rochester, New York; 2-propanol from Aldrich, Milwaukee, Wisconsin; solvents for high performance liquid chromatography and silica gel from J. T. Baker, Philipsburg, New Jersey; and other chromatographic solvents from Burdick and Jackson Laboratories, Muskegon, Michigan.

Fishery chemicals used in this study and their respective suppliers were as follows: GD-174 (technical), Glidden Durkee Corporation; Terramycin (technical) and erythromycin (technical), Sigma Chemical Company; Hyamine 1622 (50% liquid) and Hyamine 3500 (technical and 50% liquid), Rohm and Haas; and rhodamine B (powder) and rhodamine WT (20% aqueous solution), E. I. du Pont de Nemours Company.

Nitrosamine detection involved the use of gas chromatography (GC), high performance liquid chromatography (HPLC), and thermal energy analysis (TEA). Instrument characteristics used were those of Abidi (1982, 1984).

Before the experiments, we examined all products and reagents for possible contamination with nitrosamines, using steam distillation, extraction, ion exchange chromatography, and GC–TEA analysis procedures described by Abidi (1982).

Inspection for nitrosamine formation was conducted in water from the laboratory wells and the Black River, Wisconsin. The chemicals were allowed to interact for 24 h and samples were taken at intervals of 0.5, 2, 3, 6, 12, or 24 h, as appropriate for the compound under study. Nitrite-N was added at a rate of either 10 or 100 μg/L above its background levels. Resulting nitrite-N levels in the test solutions were 10, 20, 30, 100, 110, or 120 μg/L. Temperature was maintained at 20, 50, or 90°C and pH at 2, 5, or 7.

Whole-body homogenates of fish exposed to fishery chemicals and nitrite were checked for nitrosamine formation by tissue extraction, cleanup by column and thin-layer chromatography, and analysis by HPLC–TEA. Results of analyses were confirmed by GC–mass spectrometry (Abidi 1984).

Results and Discussion

There was no evidence of nitrosamine contamination in any of the fishery chemicals or reagents (level of detection 0.1 ng/g). Nitrosamines were not formed during reactions of nitrate with Terramycin, erythromycin, or the Hyamines. Forced reactions of three compounds – GD-174 and rhodamines B and WT – resulted in the formation of traces of nitrosamines under rigorous laboratory conditions of low pH and high temperature (Table 1), but no nitrosamines were found under conditions that normally occur in the natural environment. S. M. Johnson and T. R. Steinheimer (paper read at the American Chemical Society national meeting, 1984) also found that nitrosamines were not formed with rhodamine WT during simulated and actual field studies. The rate of nitrosamine formation from all three compounds was significantly greater at pH 5.0 than at pH 7.0. However, the most important factor that governed nitrosamine formation was the concentration of nitrite present.

A new nitrosamine derived from GD-174, isolated and identified as *N*-nitrosogeranylethanolamine by S. L. Abidi (paper read at the American Chemical Society national meeting, 1980), was observed in whole-body homogenates of fish treated with GD-174, but only at low concentrations (<0.5 ng/g).

Table 1. – Formation of nitrosamines from reactions of fishery chemicals with nitrite. Reaction conditions were: nitrite : amine ratio, 3; temperature, 90°C; pH, 4.5; reaction preiod, 2 h.

Fishery chemical	Yield (g/mole amine)[a]	Nitrosamine formed
GD-174	18.1	*N*-nitrosogeranylethanolamine
Terramycin	ND	
Erythromycin	ND	
Hyamine 1622	ND	
Hyamine 3500	ND	
Rhodamine B	1.9	Diethylnitrosamine
Rhodamine WT	3.0	Diethylnitrosamine

[a] ND = None detected (limit of detection 0.1 ng/g).

Of the chemicals evaluated, three produced trace concentrations of nitrosamines only when the medium was highly enriched with nitrite-N at more than 100 µg/L. Concentrations of nitrite that were required for the formation of nitrosamines are unlikely to occur in nature because nitrite is unstable in natural water systems and is readily oxidized to nitrate by nitrifying bacteria. Waters with concentrations of nitrite high enough to allow nitrosamine formation would be considered heavily polluted and unacceptable for discharge, culture waters, or potable waters. Also, the conditions of high temperature (90°C) and low pH needed to form nitrosamines are not likely to be present in natural or fish culture water systems. We conclude that the controlled use of the seven fishery chemicals in natural environments will not lead to the formation of nitrosamines.

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TECHNICAL NOTES

Use of Demand Feeders on Large Steelhead Rearing Ponds

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Abstract.—Demand feeders were installed on 5-acre rearing ponds for fingerling steelhead (*Salmo gairdneri*) to replace truck-mounted feed blowers. Because the demand feeders could dispense dry feed, substantial savings in feed costs were realized. The demand feeders supported acceptable fish survival and feed conversion rates, and they removed the uncertainty of feed calculations associated with large populations that cannot be monitored between stocking and harvest.

Problems with feed conversions ranging up to 173:1 and averaging about 3:1 (pounds of feed fed : pounds of weight gained) were encountered in four 5-acre steelhead (*Salmo gairdneri*) and sea-run cutthroat trout (*Salmo clarki*) rearing ponds at the Cowlitz Trout Hatchery (Tipping et al. 1985). Large pond size precluded accurate estimates of fish numbers until the ponds were drained. Losses from disease and predation (commonly 30–70%) made calculations based on estimated fish population sizes difficult to compute and subject to error.

The 5-acre ponds are 165 ft wide and 1,450 ft long; water flows to each pond are about 8 ft^3/s. Steelhead fingerlings typically are stocked in ponds during summer and harvested the following spring. Until 1984, the standard feeding method involved a blower feeder mounted on a truck. Oregon Moist Pellet trout diet (not more than 35% moisture) was dispensed several times a day at recommended chart rates as the truck was driven around the ponds.

Based on the success of Babington demand feeders in raceways described by Statler (1982) and Kindschi (1984), demand feeders were installed on one Cowlitz hatchery pond in 1983 and on the remaining three ponds in 1984. Briefly, a Babington feeder is a covered cylinder containing dry feed that is mounted above the water. A metal rod from the bottom of the feeder extends into the water; when tapped by fish, it releases a small amount of feed.

In 1983, about 350,000 steelhead fry, averaging 100 fish/lb, were acclimated to demand feeders in raceways. They were transferred to one 5-acre pond equipped with demand feeders when they averaged about 40 fish/lb in July and August. One 125-lb-capacity demand feeder was placed on each of eight docks that extended 30 ft from shore. The feeders were about 300 ft apart around the shore and about 200 ft apart across the pond. Closed-formula dry diet (less than 10% moisture) was used and feeders were kept full at all times. Two other ponds were stocked at similar densities and fish were fed Oregon Moist Pellets with the blower feeder. Fish were harvested from all three ponds in April and May 1984, when survival and feed conversion were calculated. In 1984, four ponds were equipped with demand feeders. They were stocked with 250,000–448,000 fingerlings each during summer and harvested in spring 1985.

The 1984 harvest indicated that survival and size were similar for steelhead fed from demand and blower feeders, but fish served by demand

TABLE 1.—Survival, mean length, and feed conversion of steelhead fingerlings fed by demand feeder or blower feeder in 5-acre rearing ponds in the 1983–1984 season.

Feeder type and pond	Survival, percent	Length, inches (N)	Feed conversion[a]
Blower			
Pond 1	66.3	8.1 (284)	2.28
Pond 2	69.7	8.0 (390)	2.16
Demand			
Pond 4	67.2	8.4 (420)	1.84

[a] Weight of feed fed ÷ weight gained by surviving fish.

TABLE 2.—Average survival and feed conversion of steelhead fingerlings reared in 5-acre ponds at Cowlitz Trout Hatchery, Washington, 1980–1985.

Harvest year	Feeder	Survival, percent	Feed conversion[a]
1980	Blower	52.2	2.41
1981	Blower	51.2	2.63
1982	Blower	32.7	6.23
1983	Blower	48.9	2.71
1984	Blower	66.0	2.04
1985	Demand	71.9	1.89

[a] Weight of feed fed ÷ weight gained by surviving fish.

feeders had the better feed conversions (Table 1). The 1985 harvest indicated higher survival and lower feed conversions associated with demand feeders than had occurred in previous years when blowers were used (Table 2).

Because two different diets and two methods of feeding were used, it cannot be concluded that demand feeders alone resulted in better feed conversion. However, dry diet costs about 40% less per pound and, with the feed conversions observed, an annual savings of about 50% (or $50,000) for all four ponds has been realized. Demand feeders also reduce personnel requirements because the feeders had to be filled an average of less than once a day.

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Occurrence of the Noninfective Stage of *Ceratomyxa shasta* in Mature Summer Chinook Salmon in the South Fork Salmon River, Idaho

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Abstract.—This report documents the occurrence of *Ceratomyxa shasta* in mature summer chinook salmon (*Oncorhynchus tshawytscha*) in the South Fork Salmon River, Idaho, in 1984 and notes the parasite's possible contribution to prespawning mortalities in this stock of fish. This represents an extension of the range of the noninfective stage of this parasite.

Ceratomyxa shasta, a myxosporidian parasite of wild and cultured salmonids, is known to occur in certain drainages in the Pacific northwest. The parasite has been reported in fish from California, Oregon, Washington, Idaho, and British Columbia; however, the infective stage has not been shown to occur in all of these areas (Coley 1979; Johnson et al. 1979; McDonald 1983). Infected fish observed in the Columbia River drainage above the Deschutes River in Oregon have been limited to migrant adult anadromous salmonids that presumably were exposed to the infective stage during their upstream migration through the lower Columbia River (Sanders et al. 1970; Johnson et al. 1979). Areas in Idaho where *C. shasta* has been observed in adult anadromous salmonids (Figure 1) are limited to the Snake River as far upstream as Oxbow Dam (Sanders et al. 1970), the North Fork Clearwater River to Dworshak Dam, the Clearwater River to Kooskia National Fish Hatchery (Joe Lientz, U.S. Fish and Wildlife Service, personal communication), the Salmon River below the confluence of the South Fork Salmon River (Johnson et al. 1979), and the Rapid River to Rapid River Hatchery (Coley 1979).

This report documents the observation of *C. shasta* in mature summer chinook salmon (*Oncorhynchus tshawytscha*) from Idaho's South Fork Salmon River in 1984, which represents an extension in the range of the noninfective stage of this parasite, and notes the parasite's possible contribution to prespawning mortalities experienced by this stock of fish.

During 1984, intestines from 122 3-, 4-, and 5-year-old summer chinook salmon, trapped for spawn-taking purposes from the South Fork Salmon River, 118 km upstream from its confluence with the Salmon River, were collected and either refrigerated, frozen, or fixed in Bouin's fluid. Fish that had died prior to spawn taking or that were killed prior to spawn taking and given to a local Indian tribe and fish that were killed during spawn taking were sampled. Refrigerated samples were examined on the day of collection while the frozen and the fixed samples were examined at a later date. Microscopic examinations under 100× and

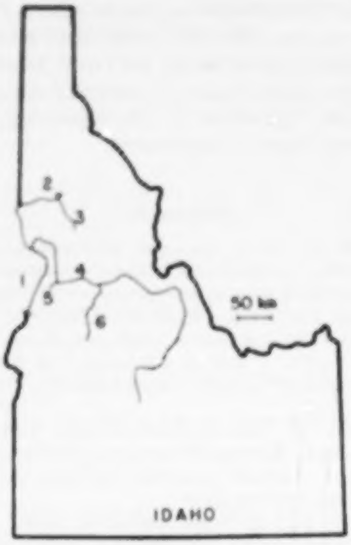

FIGURE 1. — Waters in Idaho containing anadromous salmonids observed to be infected with spores of *Ceratomyxa shasta*. Legend: (1) Snake River; (2) North Fork Clearwater River; (3) Clearwater River; (4) Salmon River; (5) Rapid River; and (6) South Fork Salmon River.

400× power were made of unstained wet-mounted scrapings of the lower intestine, and the presence or absence of spores of *C. shasta* was recorded.

Thirty-six percent of all fish examined were infected with spores of *C. shasta*; however, a much higher percentage of fish that had died prior to spawn taking were infected than fish that were killed for giveaway or spawn taking (Table 1). Greater numbers of spores were usually observed in samples taken from fish that died prior to spawning than from fish killed during hatchery operations. Infection prevalence was not constant throughout the sampling period in fish dying prior to spawning. Only 33% (eight of 24) of the fish dying prior to August 21 were infected while 91% (20 of 22) of those dying after that date were infected.

The detection of *C. shasta* spores in mature chinook salmon in the South Fork Salmon River has not been reported previously and this detection extends the known range of occurrence of the noninfective stage of this parasite. All anadromous salmonids migrating into Idaho, however, must migrate through the lower Columbia River and may be exposed to the infective stage of *C. shasta*. All areas of the state accessible to anadromous salmonids, therefore, are likely to contain fish infected with *C. shasta*. Because the infective stage of this parasite is not known to occur in Idaho and because no juvenile salmonids have reportedly become infected, even in areas containing mature salmonids harboring spores of this parasite, little danger of infection in juveniles apparently exists in Idaho.

TABLE 1. — Prevalence of *Ceratomyxa shasta* spores in mature summer chinook salmon from the South Fork Salmon River, Idaho.

Circumstances of death	Number of fish: Examined	Number of fish: Containing spores	Percent infected
Killed for giveaway or spawn taking	76	14	18
Died prior to spawning	46	30	65
Total	122	44	36

Ceratomyxa shasta infection can contribute significantly to prespawning mortality of anadromous salmonids (Sanders et al. 1970; Johnson et al. 1979). Coley (1979), however, believed that water temperatures at Rapid River hatchery, Idaho, were low enough in most years to suppress the development of the parasite and prevent parasite-induced mortality of spring chinook salmon held there. Data presented here contradict that conclusion. Water temperatures in the South Fork Salmon River are similar to those of the Rapid River, with the average daily water temperature during the holding period usually exceeding 12°C (Bill Hutchinson, Idaho Department of Fish and Game, personal communication). In this study, fish dying prior to spawning were infected more often and with a larger number of spores than were fish killed during hatchery operations. The cause of death of prespawning fish was not definitely determined to be due to *C. shasta* in this study, but it is reasonable to surmise that *C. shasta* played a contributing role. Other factors, notably bacterial kidney disease, contributed to the death of at least some of these fish.

There is further support for the conclusion that *C. shasta* contributed to prespawning mortality of fish from the South Fork Salmon River. Nearly three times more of the prespawning fish that died after August 21 were infected than were those that died before that date. Because fewer of the fish that died early in this study were obviously infected than were those that died later, it is likely that, at the temperatures experienced in 1984, development of the parasite in the fish dying early usually was not advanced enough to allow detection of the spore stage. Most of the later mortalities occurred among fish infected with large numbers of spores and death may have occurred when sufficient development of the parasite had been achieved to cause or contribute to the death of the fish.

While it is likely that temperature affects the development of *C. shasta* in adult salmonids in a manner similar to that shown in juveniles, no published reports addressing this relationship could be found. Coley (1979) assumed temperatures in excess of 10°C were necessary for *C. shasta* to develop and contribute to prespawning mortality at the Rapid River hatchery. However, Udey et al. (1975) found that suppression of ceratomyxosis in juvenile coho salmon (*Oncorhynchus kisutch*) occurred only at temperatures below 6.7°C; above that temperature mortality from *C. shasta* occurred. Idaho chinook salmon migrate through waters shown to harbor the infective stage of *C. shasta* and the fish are held for long periods prior to spawning at water temperatures conducive to the development of *C. shasta*. It appears that some mature summer chinook salmon in the South Fork Salmon River died prior to spawning as a result of infection by this parasite.

Acknowledgments.—Personnel of the McCall Summer Chinook Salmon Hatchery assisted in the collection of samples and Charlie Smith confirmed the identification of spores. Joe Lientz, Steve Huffaker, and Ellen Oman reviewed the manuscript and made suggestions for improvements. Their assistance is greatly appreciated.

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Effect of Feed Color on Feed Acceptance and Growth of Walleye Fingerlings[1,2]

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Abstract.—Groups of walleye (*Stizostedion vitreum vitreum*) fingerlings (4.0 ± 0.3 cm total length), reared in water-upwelling units, were converted from a live diet to a red, yellow, or green, semipurified, soft diet over a 10-d period. Results of this experiment suggest that a high percentage (up to 90%) of small walleye fingerlings can be converted from live food to a semipurified diet whose color contrasts with the color of the rearing container.

Hatchery biologists have had limited success in intensive culture of large numbers of walleye (*Stizostedion vitreum vitreum*) fry and fingerlings (Nickum 1978). A major constraint on the development of successful intensive-culture techniques for walleyes has been the high rate of mortality occurring when fry are started on, or fingerlings are converted to, artificial feeds. The highest survival observed when fingerling walleyes are fed artificial feeds has been in the range of 40–60% (Cheshire and Steele 1972; Nagel 1976; Beyerle

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[1] Michigan Agricultural Experiment Station Article 11572.

[2] Funds for this research were provided by the Michigan Agricultural Experiment Station and the Michigan Sea Grant College Program.

TABLE 1.—Composition of the semipurified experimental diet fed to walleye fingerlings.

Ingredient	Percent of diet
Casein	36.68
Gelatin	9.90
Dextrin	28.20
Alpha-cellulose	0.72
Vitamin mix[a]	0.33
Choline chloride	0.43
Mineral mix[b]	4.00
Soybean oil	10.00
Cod-liver oil	5.00
Water insoluble food coloring[c]	0.33

[a] National Research Council (1973) vitamin mixture for purified fish diets.

[b] National Research Council (1973) mineral mixture for purified fish diets.

[c] Aluminum Lake (AL) Red #3, AL Yellow #5, or AL Green #3.

1979; Reinitz and Austin 1980). The high mortality levels have apparently occurred from lack of feed acceptance. Development of an artificial feed acceptable to young walleyes would greatly increase the efficiency of walleye production in hatcheries.

Because walleyes are primarily sight feeders (Mathias and Li 1982), feed color may have a significant effect on feed acceptance. No differences were observed in acceptance of red, brown, or yellow diet particles when hard pelleted diet was fed to walleye fry (Nickum 1978); however, acceptance of that diet may have been limited due to diet texture. This study was designed to determine if diet color would improve feed acceptance and survival of walleye fingerlings fed a soft semipurified diet.

Three triplicate groups of 15 walleye fingerlings (4.0 ± 0.3 cm total length) were converted from a live diet to a red, yellow, or green semipurified diet (particle size 0.5-mm diameter or less) over a 10-d period (Table 1). Red and yellow were chosen because they represent the colors of zooplankters that are common food items in juvenile walleye diets. Green was chosen because it would mimic the color of algae. During the 10-d feed-conversion period, the amount of zooplankton fed was reduced by approximately 10% while the amount of semipurified feed fed was increased by 10% daily. A fourth triplicate group was fed a reference diet of zooplankton, primarily *Daphnia* spp. and *Chaoborus* larvae, throughout the 41-d experiment. Fish were fed in excess four times per day at 3-h intervals during the light phase of a 12-h light : 12-h dark photoperiod.

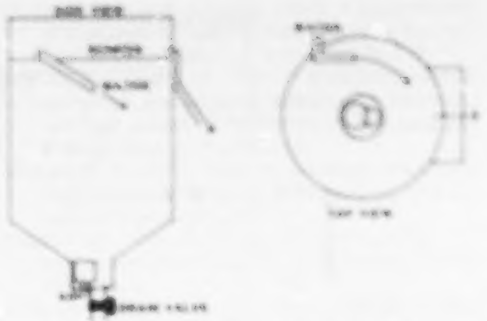

FIGURE 1.—Diagram of a rearing unit with an upwelling water-flow pattern used to raise walleye fingerlings.

Walleyes were reared in specially designed water-upwelling units. Each unit was supplied with heated well water at a temperature of 18.6 ± 0.5°C and a flow rate of approximately 0.25 L/min. To prevent gas supersaturation, the water was passed through a packed column. Excess feed and feces were drained from each experimental unit twice weekly. Lighting was supplied by a wide-spectrum fluorescent light which peaks in red-orange wavelengths (General Electric F 40 PL/AC) at an intensity of 6.3 $\mu E/m^2 \cdot s$ or less.

The water-upwelling units were made by cutting the bottoms off 4-L amber-colored glass jugs and inverting the jugs (Figure 1). Amber-colored glass jugs were used to minimize any stress that might have been caused by personnel movement around the system. Two holes were cut in a rubber stopper, which was placed in the spout of the inverted jug. One stopper opening was made to accommodate tubing for an air line and an air-stone was attached to the tubing on the internal portion of the stopper. The other stopper opening was made slightly larger to accommodate a plastic drain tube. A short piece of flexible Tygon tubing with a clamp was attached to the external part of the drain tube. A section of rigid plastic tubing was bent to an oblique angle and glued to the top of the unit to direct incoming water in a counterclockwise direction. Feed particles that sank to the bottom were drawn to the center of the unit by the counterclockwise water current, lifted by the upwelling air bubbles, and resuspended in the water column. Fiberglass screen was glued along the outer circumference of the top of each inverted jug. Effluent water flowed through the screen at the top of the unit.

When the experiment was concluded, a two-way analysis of variance was performed to test for in-

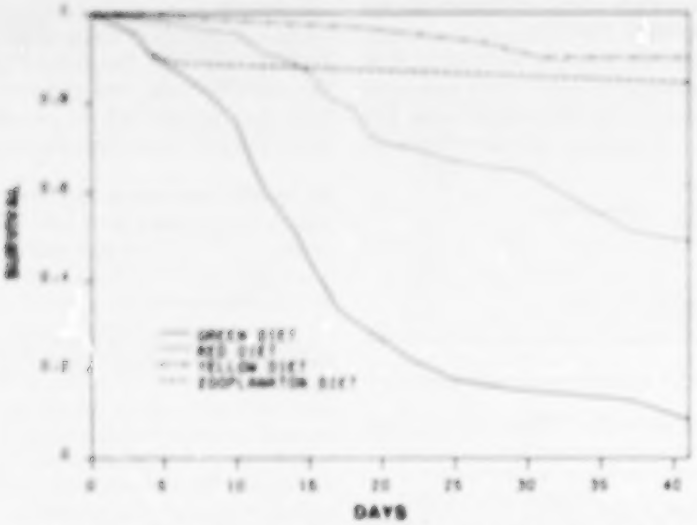

FIGURE 2.—Average survival of walleye fingerlings fed zooplankton or a yellow, red, or green semipurified diet.

teractions between each treatment. A type I probability of error of 0.05 or less ($P \leq 0.05$) was considered statistically significant. When differences occurred, comparisons were made by means of Scheffé's test (Scheffé 1959).

Analysis of the data revealed significant differences ($P < 0.05$) in survival of the fingerlings fed different colored artificial diets. There was no significant difference ($P > 0.05$) between survival of fingerlings fed zooplankton and those fed a yellow artificial diet. Survival was significantly greater ($P < 0.05$) for fish fed yellow diets or zooplankters compared to fish fed red or green diets. Percent survival (±SD) at day 41 averaged 90.0 (3.3) for fish fed yellow diet, 84.5 (3.9) for fish fed zooplankton, 48.9 (13.9) for fish fed red diet, and 8.9 (10.2) for fish fed green diet (Figure 2). During the first 25 d of the experiment 91.6% of all moribund fish were emaciated, which suggested a lack of acceptance of the colored semipurified diet. After day 25, moribund fish were not emaciated and it was assumed that all living fish had made the conversion to an artificial diet.

In our experimental environment, the interior of the units was dark except for overhead lighting. Therefore, the yellow diet would have had greater contrast than the red or green diets. Natural diet contrast has been shown to be important in the feeding of many carnivorous fishes. Pumpkinseed (*Lepomis gibbosus*) and yellow perch (*Perca flavescens*) preyed selectively on ephippeal daphnia, presumably due to greater visibility of daphnia with ephippia (Mellors 1975). Higher contrast between food color and background color increased the preference for the food when different colored trout eggs were offered to rainbow trout (*Salmo gairdneri*; Ginetz and Larkin 1973). If a neutral- or dark-colored rearing unit is desired (Nickum 1978), then feeding a practical diet that greatly contrasts with the surrounding environment should increase acceptance of the diet by fingerling walleyes.

Percent relative growth was not significantly different ($P > 0.05$) between fish fed any of the semipurified diets or the zooplankton reference diet. Relative growth per day (±SD) was 2.8% (0.84) for fish fed zooplankton and 3.33% (0.39), 3.36% (1.59), and 4.01% (0.49) for fish fed yellow, red, and green semipurified diets, respectively.

There was only one observed mortality that could be directly attributed to cannibalism; it occurred in a red diet replicate. However, there were four suspected incidences of cannibalism; one fish in each of two green diet replicates and two fish in a zooplankton replicate could not be accounted for and were assumed to have been cannibalized. Cannibalism is considered an inherent characteristic of walleyes (Eschmeyer 1950). The fingerlings in the green artificial diet replicates that did not make the transition from feeding on live food to feeding on the artificial diet were starving and would have been easy prey for healthier, more aggressive fingerlings. Unaccounted mortalities in the zooplankton diet replicate may have been the result of increased competition between larger and smaller individuals. Smaller individuals more readily fall prey to cannibalism.

Results of this experiment suggest that a high percentage (up to 90%) of small walleye fingerlings, reared in an upwelling-water system, can be converted, with a high rate of survival, from live food to a semipurified diet whose color contrasts with the color of the rearing container.

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Symbols and Abbreviations

The following symbols and abbreviations, as well as others approved for the Système International d'Unités (SI), are used in this journal without definition. Any others must be defined in the text at first mention, as well as in the captions or footnotes of tables and in figures or figure captions.

Prefixes

kilo (10^3)	k
milli (10^{-3})	m
micro (10^{-6})	μ

Time and Temperature

day	d
degrees Celsius ([°F − 32]/1.8)	°C
degrees Fahrenheit ([1.8 × °C] + 32)	°F
hour (spell out for diel time)	h
minute	min
second	s

Spell out year, month, and week.

Weights and Measures (Metric)

centimeter (0.394 in)	cm
gram (0.0353 oz)	g
kilogram (2.20 lb)	kg
kilometer (0.622 mi)	km
liter (0.264 gal, 1.06 qt) (exception to SI)	L
meter (1.09 yd, 3.28 ft, 39.4 in)	m

Weights and Measures (English)

cubic feet per second (0.0283 m^3/s)	ft^3/s
foot (30.5 cm)	ft
gallon (3.79 L)	gal
inch (2.54 cm)	in
mile (1.61 km)	mi
ounce (28.4 g)	oz
pound (0.454 kg, 454 g)	lb
quart (0.946 L)	qt
yard (0.914 m, 91.4 cm)	yd

Spell out acre (0.405 hectare), hectare (2.47 acres), ton (2,000 lb, 907 kg), and tonne (1,000 kg, 2,200 lb).

Mathematics and Statistics

all standard mathematical signs, symbols, and abbreviations	
base of natural logarithm	*e*
common test statistics (*F*, *t*, etc.)	
correlation coefficient (multiple)	*R*
correlation coefficient (simple)	*r*
degree (angular or temperature)	°
degrees of freedom	df
logarithm (specify base)	log
minute (angular)	′
not significant	NS
percent	%
probability	*P*
sample size	*N*
second (angular)	″
standard deviation	SD
standard error	SE

Physics and Chemistry

all atomic symbols	
alternating current	AC
ampere	A
British thermal unit (1,055 J)	Btu
chemical acronyms listed in Webster's dictionaries (DDT, EDTA, etc.)	
direct current	DC
footcandle (0.0929 lx)	fc
hertz	Hz
hydrogen ion activity (negative log of)	pH
joule (0.239 gram-calories, 0.000948 Btu)	J
lux (10.8 fc)	lx
molar	M
mole	mol
ohm	Ω
ortho	*o*
para	*p*
pascal	Pa
parts per million (per 10^6) (in the metric system, use mg/L, mg/kg, etc.)	ppm
parts per thousand (per 10^3)	ppt, ‰
siemens (=mho, Ω^{-1})	S
volt	V
watt	W

Calories should be converted to joules in the metric system.

General (Some Are Restricted)

compass directions (maps and coordinates): east	E
north	N
south	S
west	W
District of Columbia	D.C.
et alii	et al.
et cetera	etc.
for example	e.g.,
months (tables, figures): first three letters (Feb, Jun, etc.)	
sex (tables, figures, hybrid crosses): female	♀
male	♂
that is	i.e.,
Union of Soviet Socialist Republics	USSR
United States (adjective)	U.S.
United States of America (noun)	USA

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